THE ROLE OF NATRIURETIC AGENTS IN THE MECHANISM OF HYPERTENSION IN THE DAHL STRAIN OF SALT-SENSITIVE AND SALT-RESISTANT RATS

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ABSTRACT

Title of Dissertation: The role of natriuretic agents in the mechanism of hypertension in the Dahl strain of salt-sensitive and salt-resistant rats

William Timothy Link, Doctor of Philosophy, 1986

Dissertation directed by: Motilal B. Pamnani, M.D., Ph.D.
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Previous studies have suggested that elevated salt intake and altered levels of two agents, namely: 1) a ouabain-like humoral factor (OLHF) and 2) Atrial Natriuretic Factor (ANF) may be involved in the pathogenesis of volume-dependent hypertension. OLHF, by inhibiting $Na^+ - K^+$ pump activity in cardiovascular muscle cells, stimulates the cardiovascular system, and is therefore pro-hypertensive. ANF produces natriuresis, diuresis and antagonizes vasoconstrictors, and is therefore anti-hypertensive.

In the present study, I examined whether the levels of these agents were altered in hypertension in Dahl salt-sensitive (DS) rats. DS and Dahl salt-resistant (DR) rats were fed high (8.0%) salt (HS) or low (0.4%) salt (LS) diets for six weeks. Only DS rats on HS (DS_{HS}) developed hypertension. DS rats on LS (DS_{LS}) and DR rats on HS (DR_{HS}) and LS (DR_{LS}) remained normotensive. At the end of this dietary regimen, blood and atria were collected for measurement of OLHF and ANF respectively. Inhibition of ouabain-sensitive ^{86}Rb uptake (a measure of $Na^+ - K^+$ pump activity) in tail arteries of normal rats, when exposed to plasma supernates from Dahl rats, was used as an assay for OLHF. ANF in atrial tissue extracts (AE) from Dahl rats was evaluated by bioassay in normal rats, and by radioimmunoassay. Bioassays used were: 1) urine

excretion, 2) sodium excretion and 3) inhibition of vasoconstriction in rat aortic rings. In further bioassay studies, responsiveness of DS and DR rats to AE from normal rats was determined.

The results of this study show that plasma OLHF levels are not different among the four groups of Dahl rats. However, atrial ANF levels, as determined by all assay methods, were higher in hypertensive DS_{HS} rats relative to DR_{HS} control rats. In addition, the renal excretory and vascular contractile responsiveness of DS rats to ANF was decreased relative to DR rats. These findings suggest that OLHF is not involved in the pathogenesis of hypertension in DS_{HS} rats. These findings also suggest that, possibly due to decreased renal and cardiovascular responsiveness of DS rats to ANF, the increased levels of ANF may be a compensatory response and may attenuate, but fail to prevent, hypertension in these rats.

THE ROLE OF NATRIURETIC AGENTS IN THE MECHANISM OF HYPERTENSION IN THE DAHL STRAIN OF SALT-SENSITIVE AND SALT-RESISTANT RATS

by

William Timothy Link

Dissertation submitted to the Faculty of the Department of Physiology
Graduate Program of the Uniformed Services University of the
Health Sciences in partial fulfillment of the
requirements for the degree of
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DEDICATION

To my family and all my friends,

especially my parents Roger and Virginia,

and to Dina,

a very special friend and companion.

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INTRODUCTION

It is estimated today that one out of every four Americans has hypertension, with one in eight being seriously affected. Untreated, this can lead to stroke, heart failure or kidney disease. The two principle determinants of mean arterial blood pressure are cardiac output and the total peripheral resistance to blood flow. In most established cases of hypertension, the elevated systemic arterial pressure can be shown to be maintained by increased vascular resistance to blood flow while cardiac output is normal or even low. Effective treatment necessitates a knowledge of the etiology of this increased vascular resistance, but only ten percent of these patients have hypertension of a known cause, termed secondary hypertension. The remainder have primary, or essential, hypertension where the underlying cause is not known.

Recent evidence suggests that body fluid volume and its regulation play a central role in the genesis and maintenance of arterial hypertension. Investigations of animal models of hypertension have aided in clarifying some of the physiological alterations in the regulation of body fluid volumes that occur in the hypertensive process. These models include those where surgical or pharmacologic manipulations are performed in order to compromise normal fluid volume maintenance, or are the result of genetic inbreeding. The pathophysiology of the increased vascular resistance cannot be explained solely on the basis of alterations in known vasoactive agents, although some of these agents play varying roles in experimental models and contribute more in some

models than others.

Several hypotheses have been suggested to explain the mechanism of this elevated peripheral resistance to blood flow. One that has gained much popularity concerns the role of dietary sodium chloride intake in the development and maintenance of hypertension. Evidence for this hypothesis comes from experimental, clinical and epidemiological studies.

THE ROLE OF SALT IN HYPERTENSION

Sapirstein et al. (1950) studied the relationship between systolic blood pressure and salt intake in rats by substituting sodium chloride solutions for normal drinking water. Arterial hypertension was reported, which developed rapidly following a latent period of one to four weeks of saline drinking.

Meneely et al. (1953) extended these studies using a larger range of sodium chloride concentrations. The concentrations tested were: 0.01, 0.15, 2.8, 5.6, 7.0, 8.4, and 9.8 percent. Significant elevations in mean systolic blood pressure were found in all groups drinking 2.8 to 9.8 percent sodium chloride solutions. Furthermore, a linear relationship between blood pressure and salt intake was demonstrated following nine months on the dietary regimen.

Dahl (1961) demonstrated the induction of hypertension in rats eating 8.0 percent sodium chloride chow. Interestingly, in the majority of these hypertensive rats, their hypertension became self-sustaining. After one year of high salt intake, two thirds of these rats failed to show significant reductions of blood pressure in response to withdrawal

of high salt from their diet. The remaining one third showed some reduction of blood pressure and a small percentage of these became normotensive again. The wide variation in individual responses was utilized by Dahl to obtain two strains of rats differing only in their responses to dietary salt intake. (These strains of rats are discussed in greater detail below under the heading of "The Dahl strain of salt sensitive and salt resistant rats").

Studies in dogs also suggest a role of salt in the pathophysiology of hypertension. Vogel (1966) reported the induction of arterial hypertension in dogs by substituting 0.9% NaCl for normal drinking water and increasing their dietary NaCl in food by 2g/kg body weight per day. Arterial pressure increased 55% over a period of 26 days. In a second group of dogs, aortic catheters were chronically implanted prior to salt feeding for recording blood pressure in the conscious unrestrained state. The elevation of blood pressure was reported to be due to increased cardiac output attributed to increased stroke volume rather than heart rate. Calculated peripheral resistance was found to be not altered. Blood and plasma volume were significantly increased, and hematocrit significantly decreased, following 9 and 24 days of increased dietary salt intake.

Clinical studies monitoring blood pressure responses to restriction of dietary salt intake provide further evidence for a role of sodium chloride in hypertension.

Kempner (1948) studied the blood pressure responses in 500 human patients with hypertensive vascular disease following treatment with a rice diet containing not more than 300 mg of sodium chloride. The patients consumed 250 to 350 grams (dry weight) of rice steamed in water

or fruit juice daily. Of the original 500 patients, 322 showed improvement by an average reduction of blood pressure of 20 mmHg and a reduction in heart size of 18% or more. Twenty five percent of these 500 patients became normotensive again. The length of time required for the blood pressure to decrease varied from four days to 10 months. It was suggested by the investigator that the remaining patients may have had extensive vascular complications that were irreversible.

Murphy (1949) extended these studies using the Kempner rice diet on 17 patients and monitored plasma and interstitial fluid volumes by dye dilution techniques. Patients were monitored for 100 days. Mean decreases in plasma volume and interstitial fluid volume of 10% and 12% respectively were reported. Significant blood pressure reductions were noted in 16 of the 17 patients.

Miller et al. (1983) studied the blood pressure responses to dietary sodium restriction in 16 healthy, normotensive husband-wife pairs. Sodium intake was restricted to less than 60 mEq/day for a period of 12 weeks. Significant decreases in both systolic and diastolic blood pressure were reported for both men and women at the end of the test period. The authors suggested that the blood pressure response to sodium restriction may not be limited to hypertensives and that the response is heterogeneous in normotensive subjects, with certain individuals showing greater responses than others.

The above studies demonstrate that sodium restriction can lower blood pressure in some hypertensive patients. In addition, the studies below show drugs that cause increased excretion of salt and water, such as thiazide diuretics, are also associated with a lowering of blood pressure in hypertensives.

Wilson and Freis (1959) reported significant reductions in blood pressure, plasma and extracellular fluid volumes in hypertensive patients treated with chlorothiazide. These reductions occurred within the first 48 hours of treatment. Furthermore, in six of these patients, acute restoration of plasma volume with 6% dextran in isotonic saline resulted in immediate elevation of blood pressure an average of 15 mmHg.

Frohlich et al. (1960) measured the hemodynamic responses to short term chlorothiazide treatment. Catheters were placed in the right atria of seven hypertensive patients and control cardiac output and direct blood pressure determinations were made. Following three days of chlorothiazide (1500 mg/day) treatment, cardiac output, plasma volume and blood pressure were measured. Systolic and diastolic blood pressure fell in all patients, averaging 17% and 13% respectively. Cardiac output was decreased an average of 29% from control values, and plasma volume was decreased by 13%.

The above studies show that the short term effect of diuretic treatment is a fall in blood pressure caused by reduction of cardiac output. Conway and Lauwers (1960) confirmed these results in another clinical study where the chlorothiazide treatment was continued for an average period of 5 to 6 months. Initially, following one to two weeks of treatment, plasma volume, blood pressure and cardiac output were reduced. However, following one month of treatment, cardiac output and plasma volume returned to pretreatment levels while the fall in blood pressure was maintained by a reduction in the total peripheral resistance.

The above clinical studies demonstrate that reduction of body fluid volumes is associated with decreased blood pressure and peripheral

resistance in a large percentage of hypertensive individuals, and that the decrease in blood and extracellular fluid volume precedes the decrease in peripheral vascular resistance. Calculated peripheral resistance actually increases initially following the reduction of blood and extracellular fluid volume, probably a response similar to that observed during hemorrhage, and the blood pressure depression observed during the initial three weeks of diuretic therapy is at the expense of blood flow to the tissues.

The above studies suggest that excess salt is associated with increased blood pressure while salt depletion is associated with decreased blood pressure. Epidemiological studies have further strengthened this hypothesis.

Prior et al. (1968) studied the blood pressure and dietary salt intake of two Polynesian populations. In the Rarotonga group, blood pressure increased with age, especially in women, while in the Pukapuka group this increase is only slight and seen only in women. It was determined through dietary surveys and 48 hour urine collections that the average sodium intake of the Rarotonga group was 50 mEq/day higher than that of the Pukapuka group, again suggesting a relationship between salt intake and the average blood pressure level of a given population group.

Page et al. (1974) studied possible cardiovascular and dietary interactions in six Solomon Islands societies. The societies were ranked as to their degree of acculturation based on contact with Western cultural influences, education, availability of medical care, economy and diet. Systolic blood pressure was found to increase with age in females of the three societies with the highest degree of acculturation,

while this trend was absent in the groups with the lowest cultural ranking. Diastolic blood pressure in males fell with age in the less accultured groups, but this was not apparent in the groups with the highest cultural ranking. The trends in blood pressure, in relation to age, were found to correlate best with diet, especially in the intake of salt, tinned meat and fish.

Oliver et al. (1975) studied blood pressure, salt intake and urinary sodium excretion in the South American Yanomamo Indians who do not use salt in their diet. In contrast to the trend in civilized populations, no systematic increase in blood pressure with age was observed, again suggesting a role for salt intake in the average blood pressure of a population.

THE ROLE OF NATRIURETIC FACTORS IN HYPERTENSION

The above studies suggest that the arterial pressure of normal animals can be raised by increasing their salt intake. Furthermore, the incidence of hypertension in human populations seems to correlate with the level of their salt intake. Restriction of salt intake, or the increased excretion of salt and water in response to diuretic treatment have been shown to decrease blood pressure. Taken together, these findings provide a strong argument for a role of sodium chloride in arterial hypertension. The mechanism by which increased salt intake leads to the development of hypertension, however, remains obscure.

These effects of salt seem to be the result of increases in body fluid volume rather than a direct effect of the increased salt. Norman et al. (1975) reported only small (4 mmHg) increases in blood pressure

in unilaterally nephrectomized sheep following seven days of elevated plasma sodium when the extracellular fluid volume was held constant by water deprivation. Thus, elevation of extracellular salt without an accompanying increase in fluid volume does not cause appreciable increases in arterial blood pressure. Elevation of extracellular fluid volume, without increased extracellular salt, can however be shown to elevate blood pressure.

Manning et al. (1979) reported significantly increased mean arterial pressure, in dogs with 70% renal mass reduction, following 14 days of continuous infusion of hypotonic saline and subpressor doses of vasopressin. Arterial pressure rose after two days of treatment while, at the same time, plasma sodium was decreased.

Finnerty et al. (1970) demonstrated that intravenous infusion of five percent glucose solutions into hypertensive patients resulted in 15 to 20% increases in arterial blood pressure in 60 to 90 minutes while plasma sodium concentration was not increased.

While it seems apparent that the effect of salt on blood pressure is mediated by elevation of body fluid volumes secondary to the salt retention, the sustained increase in blood pressure seen in hypertension cannot be explained by increased volume alone. The above studies demonstrate that chronic extracellular fluid volume expansion results in sustained increases in arterial blood pressure, especially when renal handling of the volume load is compromised (such as reduction of renal mass). This response is not immediate, however, and can take hours or days to occur.

Conway (1966) measured cardiac output, arterial blood pressure and calculated vascular resistance in trained unanesthetized dogs

following expansion of blood volume with whole blood mixed with Dextran to minimize alterations in hematocrit. Cardiac output and blood pressure rose slightly, but cardiac output returned to control levels by one and one half hours. Vascular resistance decreased initially following infusion, then increased to approximately 20% above control in two hours. The elevated blood pressure was maintained by this increased vascular resistance for the remainder of the 10 hours of observation. Autonomic ganglionic blockade with pentolinium did not alter this pattern.

Coleman and Guyton (1969) reported similar findings in dogs with reduced renal mass when saline loaded by continuous isotonic saline infusion or by substitution of isotonic saline for normal drinking water. Cardiac output and arterial blood pressure were monitored for two weeks. Transient increases in cardiac output and stroke volume were reported in the first week of treatment along with increased blood pressure. During the second week cardiac output decreased while blood pressure increased further, sustained by an increase in peripheral resistance.

It therefore seems apparent that expansion of extracellular fluid volume by increased dietary salt and/or decreased renal salt-excreting capacity can lead to the development of hypertension. The mechanism behind this phenomenon is unknown. Elevated cardiac output has been implicated in the early phases, but in the established phase, the elevated arterial pressure could be shown to be due to increased vascular resistance to blood flow while cardiac output was near normal. Body fluid volumes may be normal, increased or decreased in the established phase of hypertension. In the reduced renal mass model

fluid volumes are increased (Huot et al., 1983), while in other models the findings are not clear. This may be due to absolute and/or relative changes in fluid volume and the time course of the development of the hypertension. Several hypotheses have been suggested to explain how increased body fluid volume can lead to increased vascular resistance and hypertension.

Coleman et al. (1971) suggested that the increase in vascular resistance was part of the normal autoregulatory response, delayed in onset by the nervous system via the baroreceptors. In response to the fluid volume expansion, central venous pressure, and therefore venous return to the heart, increase. Cardiac output should therefore increase in accordance with the Frank - Starling law of the heart. The authors felt that this increased cardiac output would lead to overperfusion of the tissues which, through the classic autoregulatory response, would cause peripheral vasoconstriction. The increased vascular resistance was felt to be the normal autoregulatory response to this overperfusion.

Several lines of evidence suggest that this hypothesis of "long term whole-body autoregulation" may not adequately explain the elevated vascular resistance seen in volume expanded hypertension. First, the classic autoregulatory response occurs within seconds to minutes, while the increased vascular resistance seen in volume expanded hypertension takes hours or days to develop (Haddy and Overbeck, 1976). Secondly, while it was argued that the autoregulatory response may be delayed by the baroreceptor mechanism (Coleman et al., 1971), the previously mentioned study by Conway (1966) demonstrated that autonomic blockade did not decrease the time interval for the onset of the increased vascular resistance.

Additionally, the long term whole-body autoregulation hypothesis depends on increased cardiac output as the initiating factor. However, Fletcher et al. (1976) presented evidence that hypertension can occur in the absence of significant changes in cardiac output. They measured blood pressure, cardiac output and vascular resistance during the development of renal hypertension in white New Zealand rabbits. Although increased cardiac output was observed in hypertensives, it was observed in control, sham-operated rabbits as well. It was concluded that the increased cardiac output was a nonspecific consequence of the surgical procedure. Peripheral vascular resistance was significantly increased, relative to control rabbits, within the first week and the authors concluded that the hypertension was mediated by increased resistance from its earliest stages.

Similarly, Onoyama et al. (1979) measured cardiac output and blood pressure in metyrapone-treated dogs on different levels of sodium intake relative to dogs on identical sodium intakes not receiving metyrapone. Only metyrapone-treated dogs became hypertensive, however their cardiac output was not different from control dogs fed the same sodium intake. At levels of sodium intake less than 140 mEq, vascular resistance decreased as cardiac output increased. However, at sodium intakes greater than 140 mEq resistance and blood pressure increased, although the rise in cardiac output was not greater than that observed at sodium intakes less than 140 mEq. The authors concluded that this form of hypertension was mediated by increased peripheral resistance, and that cardiac output did not play a major role in the process.

Finally, a study by Mark et al. (1975) demonstrated responses in normotensive patients that were opposite to those proposed by the

autoregulation hypothesis. In response to increased salt intake, normotensives showed decreased vascular resistance and increased forearm blood flow. Borderline hypertensives showed the opposite response: vasoconstriction and decreased forearm blood flow.

Hypertension can also occur when there is no evidence of increased vascular resistance and the hypertension can be shown to be due to elevated cardiac output alone (Tarazi et al., 1973). Under the autoregulation hypothesis, such an increase in cardiac output should result in elevated vascular resistance. Together with the above studies, this suggests that the autoregulation hypothesis may not be sufficient to fully explain the mechanisms behind the development of volume expanded hypertension.

Another hypothesis suggested to explain the increased vascular resistance seen in hypertension involves the altered concentration of a vasoactive agent or agents. Investigation of the known vasoactive agents has failed to establish a primary role for angiotensin, norepinephrine, vasopressin, kinins or prostaglandins in the pathogenesis of volume-dependent hypertension (Haddy and Overbeck, 1976). Evidence from several laboratories suggests that levels of newly described natriuretic and vasoactive substances are altered in response to volume expansion and in several models of experimental hypertension. These substances may play a role in the pathogenesis of the increased vascular resistance observed in volume expanded hypertension.

In our laboratory we have been studying the role of two natriuretic substances that seem to be involved with regulation of body fluid volume. These substances have been shown to be elevated in response to acute and chronic elevation of extracellular fluid volume

and have been implicated in several forms of experimental hypertension. Their role in salt dependent hypertension has not been extensively investigated. The first substance, as yet unidentified, has been termed a "ouabain-like humoral factor", or OLHF, in our laboratory because it is a potent inhibitor of vascular $Na^+ - K^+$ pump activity. It has also been referred to as the Natriuretic Hormone. Similar ouabain-like activity in plasma and urine has been demonstrated by several other investigators. The second substance is the newly discovered Atrial Natriuretic Factor or ANF which has become the focus of extensive research efforts in numerous laboratories.

The Ouabain-Like Humoral Factor (OLHF)

Studies from other laboratories

Natriuretic activity in the plasma and urine of volume expanded animals has been reported by several laboratories. Sealey et al. (1969) reported natriuretic activity in urine and plasma of acutely volume expanded human patients, and also in Dorset female sheep following four months of saline drinking. Plasma and urine samples were partially purified by gel filtration, concentrated and injected into Long - Evans bioassay rats with mild diabetes insipidus. Significant increases in sodium excretion were reported following injection of urine or plasma extracts from expanded sheep or humans relative to urine or plasma extracts from sodium-deprived humans or sheep drinking tap water. This response was attributed to the presence of a natriuretic agent of molecular weight greater than 5000 but less than 70,000 daltons.

Blythe et al. (1971) demonstrated blood-borne natriuretic activity in acutely volume expanded dogs. Using cross-circulation techniques, blood from one donor dog, after receiving a saline infusion equal to 15% of body weight, was circulated through a recipient dog. During volume expansion of the donor, urinary sodium excretion of the recipient dog rose an average of 39%. The donor dogs showed a much larger natriuresis, averaging 575% increase over control. In a second series of experiments, the cross-circulation technique was modified to increase the exposure of the recipient dogs kidneys to the donor blood. This modification increased sodium excretion of the recipient by 300%, a much greater response, while the response of the donor dog was decreased to 300% from the 575% response observed in the first series of experiments. These investigators concluded that a humoral natriuretic agent was released into the circulation in response to the volume expansion, and that this agent acted on the kidneys of the recipient dog to elicit a natriuretic response similar to that seen in the donor dogs.

Clarkson et al. (1976) described two natriuretic substances in urine from normal volunteers. Twenty four hour urine collections were taken from salt-deprived control volunteers and from volunteers consuming 300 mEq of sodium chloride daily for six days. Following concentration and partial purification of the urine extracts, they were assayed by intravenous injection into normal, conscious, water loaded Sprague Dawley rats. Natriuretic activity was attributed to two substances, one greater than 30,000 MW, the other less than 3000. Both substances were present in urine of salt-loaded and salt-deprived volunteers. Urine extracts from salt-loaded humans produced twice the natriuresis, in assay rats, as identically prepared extracts from salt-

depleted controls, suggesting higher urinary content of these substances following salt-loading.

The above studies demonstrate the presence, in plasma and urine, of a substance(s) with natriuretic activity that increases in response to expansion of blood and extracellular fluid volume. The chemical nature of this substance, or the mechanism through which it exerts its natriuretic effects are not clear. Inhibition of active sodium transport in the kidney, causing decreased reabsorption of filtered sodium may be involved in this action.

Favre et al. (1975) demonstrated antinatriferic (inhibition of sodium transport) activity in urine from salt-loaded dogs. Gel filtration fractions of concentrated urine from dogs, fed 258 mEq NaCl plus 0.2 mg fludrocortisone per day, consistently inhibited short circuit current in the toad bladder and caused natriuresis in the rat. Urine fractions from dogs fed 3 mEq NaCl plus fludrocortisone per day, or 90 mEq NaCl per day alone, were without this effect.

Buckalew and Nelson (1974) compared plasma ultrafiltrates prepared from acutely volume expanded dogs with those from water deprived, volume depleted dogs. Significantly greater inhibition of toad bladder short circuit current was demonstrated in responses to application of ultrafiltrates from volume expanded dogs relative to those from volume depleted dogs. The same fraction that caused this antinatriferic effect was also shown to promote natriuresis and diuresis following i.v. injection into partially nephrectomized rats.

The inhibition of active sodium transport associated with the plasma and urine extracts mentioned above could be due to inhibition of the $Na^+ - K^+$ ATPase activity coupled with the cellular $Na^+ - K^+$ pump.

Gonick et al. (1977) isolated a small molecular weight fraction (<1000), from serum of rats, by gel filtration on Sephadex G-25. Serum fractions from volume expanded rats (10% w/w over one hour with isotonic saline) were compared to those from normal rats for their inhibitory effect on $Na^+ - K^+$ ATPase activity of kidney homogenates. Fractions from volume expanded rats caused greater inhibition of $Na^+ - K^+$ ATPase activity relative to fractions from control rats. The authors proposed that a factor, similar to ouabain in its effect on $Na^+ - K^+$ ATPase, was released into the circulation in response to expansion of the extracellular fluid volume.

Gruber et al. (1980) reported the presence of similar activity in plasma ultrafiltrates from volume expanded dogs. The partially purified substance was shown to inhibit activity of purified hog brain $Na^+ - K^+$ ATPase and, furthermore, shown to bind anti-digoxin antibodies in a fashion similar to digoxin.

Poston et al. (1981) measured active sodium transport in white blood cells of essential hypertensive patients. They reported decreased ouabain-sensitive ^{22}Na efflux (a measure of active $Na^+ - K^+$ pump activity) in cells from hypertensive patients relative to those from normotensive controls. Furthermore, incubation of normal cells (from normotensive donors) in plasma from essential hypertensive patients caused a decrease in active sodium efflux from these normal cells to a level that was similar to that seen in blood cells from essential hypertensives. The authors suggested that a circulating $Na^+ - K^+$ pump inhibiting factor was present in the blood of hypertensive patients, had ouabain-like activity, and was responsible for the impaired sodium transport observed in the blood cells of these hypertensive patients.

Studies from our laboratory

Studies in our laboratory have provided similar findings in response to acute volume expansion in rats and dogs, and in several forms of low renin, volume expanded experimental hypertension (Pamnani et al., 1981). Decreased vascular $Na^+ - K^+$ pump activity, as measured by ouabain-sensitive ^{86}Rb uptake was demonstrated in mesenteric arteries from one-kidney, one-wrapped (1-K,1W) hypertensive dogs relative to pump activity in arteries from one-kidney normotensive control dogs. Similar decreases in vascular $Na^+ - K^+$ pump activity were seen in tail arteries of one-kidney, one clip hypertensive rats and in one-kidney, DOCA-salt hypertensive rats relative to pump activity in arteries from respective, normotensive control rats. Boiled plasma supernates from 1-K, 1W dogs and one-kidney, one clip hypertensive rats decreased ouabain-sensitive ^{86}Rb uptake of normal tail arteries when applied to them, suggesting that the suppressed pump activity observed in these hypertensive animals is due to the action of a plasma factor.

Huot et al. (1983) further extended these studies to another model of hypertension. They demonstrated decreased vascular $Na^+ - K^+$ pump activity, decreased cardiac microsomal $Na^+ - K^+$ ATPase activity and the presence of this ouabain-like factor in plasma supernates of rats with reduced renal mass hypertension drinking 1.0% saline relative to normotensive reduced renal mass rats drinking distilled water.

Clough et al. (1977a, 1977b) reported decreased $Na^+ - K^+$ ATPase activity in cardiac microsomal preparations of left and right cardiac ventricles of rats with one-kidney, one-clip hypertension relative to their one-kidney controls.

In contrast to the findings of decreased cardiovascular muscle

cell $Na^+ - K^+$ pump activity in several volume dependent types of experimental hypertension, increased pump activity has been reported in the genetic forms of hypertension studied so far. Pamnani et al. (1980) and Overbeck et al. (1981) both reported increased rather than decreased vascular $Na^+ - K^+$ pump activity in Dahl Salt-Sensitive "S" rats as compared to their Salt-Resistant "R" rat controls. Similar findings were reported for another genetic form of hypertension where $Na^+ - K^+$ pump activity was increased in the Spontaneously Hypertensive Rat (SHR) relative to its normotensive Wistar Kyoto (WKY) control (Pamnani et al.,1979). Plasma supernates were not investigated in these models and the significance of the observed increased pump activity is not yet understood. It may however be a compensatory response to an increase in membrane permeability to sodium ions. Such an increase in permeability has been suggested by studies in the SHR where Jones (1973) and Freidman (1979) demonstrated increased passive sodium fluxes in tail arteries of SHR relative to WKY rats.

The role of OLHF in hypertension

Decreased $Na^+ - K^+$ ATPase activity and $Na^+ - K^+$ pump activity, through the action of this circulating agent could provide an explanation for the increased vascular resistance seen in volume expanded models of hypertension. Decreased pump activity would lead to depolarization and increased Ca^{++} entry by voltage dependent channels, or through alteration of the Na^+/Ca^{++} exchange mechanism proposed by Blaustein (1977). This, in turn, could lead to vasoconstriction, increases in cardiac contractility and increased responsiveness of

vascular smooth muscle cells to vasoconstrictor agents. All of these influences could be involved in the development and maintenance of hypertension.

Atrial Natriuretic Factor (ANF)

The discovery of ANF began with the observation of specific-staining granules in atrial muscle cells of mammals (Kisch, 1955; and Jamieson and Palade, 1964). de Bold (1979) demonstrated changes in atrial granularity following alterations in water and electrolyte balance in rats. Water deprivation and sodium deficiency were associated with atrial hypergranulation, whereas treatment with Deoxycorticosterone acetate and 2% saline substituted for normal drinking water lead to atrial degranulation. A significant positive correlation between atrial granulation and hematocrit values was reported by the author.

The presence of a natriuretic and diuretic factor in atrial tissue was first demonstrated by de Bold et al. (1981). Intravenous injection of atrial, but not ventricular, tissue extracts resulted in large increases in urinary sodium and water excretion. Later studies (de Bold, 1981, and Garcia et al., 1982) demonstrated that the atrial granules were the source of this natriuretic activity. At this time, in our laboratory, we examined this factor to determine if the natriuretic factor from rat atria was the same as the ouabain-like humoral factor which was shown to be present in several forms of low renin, volume expanded experimental hypertension (Pamnani et al.,1981). Our results indicated that the two factors are not identical. Unlike the ouabain-

like factor, the atrial factor had no significant effect on vascular $Na^+ - K^+$ pump activity, cardiac microsomal $Na^+ - K^+$ ATPase activity, or short circuit current measured in the toad bladder (Pamnani et al., 1984). Trippodo et al. (1984) further confirmed these results by demonstrating that the atrial extracts did not alter ouabain-dependent sodium efflux from human erythrocytes.

Renal effects of atrial extracts

The studies of de Bold et al. (1981) clearly demonstrate the presence of a factor in atrial tissue with potent diuretic and natriuretic activity. The mechanisms of action of this factor on the kidney are not yet understood. Both renovascular and tubular modes of action have been suggested to explain the natriuresis and diuresis.

Camargo et al. (1984) measured hemodynamic and tubular function parameters, in isolated perfused rat kidneys, in response to infusion of partially purified atrial extracts. Significant increases in renal vascular resistance, glomerular filtration rate and filtration fraction were noted, as well as the normally observed natriuresis and diuresis. These atrial extract-induced changes in renal vascular and tubular parameters were inhibited by low Ca^{++} (0.2mM) or Verapamil (0.01 M). In contrast to the increase in renal vascular resistance seen in isolated kidneys, the resistance actually decreased in response to atrial extracts when the resistance was previously increased by Angiotensin II, norepinephrine or vasopressin. This may provide an explanation for their report of increased resistance in isolated kidneys which are in contrast with the findings, by other investigators mentioned below, of

decreased blood pressure and renal vascular resistance in intact animals following injection of atrial extracts. Increased resistance may be the effect in isolated kidneys in the absence of endogenous vasoconstrictor activity. When this activity is present, either in situ or produced by exogenous application of vasoconstrictors, the atrial extract may antagonize their actions and cause vascular resistance to fall. These authors concluded that the renal vascular effects of the atrial extracts could account for the diuresis and natriuresis seen. However, other investigators have demonstrated that natriuresis and diuresis can occur in response to atrial extracts in the absence of observable changes in glomerular filtration rate or renal vascular resistance.

Briggs, et al (1982), examined kidney glomerular filtration rate (GFR), single nephron GFR, and proximal tubular and Loop of Henle fluid absorption rates in response to continuous infusion of atrial extracts into male Sprague Dawley rats. A "low" dose, equivalent to the tissue content of three atria per hour and a "high" dose of six atria per hour were used. Urinary sodium and water excretion increased in dose-dependent fashion, as expected. However, at low doses, whole kidney and single nephron GFRs were unaffected in spite of increased sodium and water excretion. At the high dose, both whole kidney and single nephron GFR were significantly elevated and the natriuresis and diuresis were greater than at the low dose. The authors concluded that at the low dose the atrial extract had its effect through a distal tubular mechanism in the absence of changes in GFR. Net proximal fluid reabsorption was unaltered while distal tubular reabsorption was decreased. At the higher dose, the increase in GFR could account for the further increase in sodium and water excretion. In addition, the

authors examined the tubuloglomerular feedback response before and after atrial extract infusion at the high dose using separately perfused segments of the same nephron. Increasing the Loop of Henle flow from 0 to 40 nl/minute in the control period caused a drop of 9.1 nl/minute in early proximal flow. During high dose atrial extract infusion, the same increase in Loop flow caused a decrease of only 1.95 nl/minute in early proximal tubular flow. This blunting of the tubuloglomerular feedback response was apparently not mediated from the luminal side because perfusion of the Loop with solution containing atrial extract did not cause this attenuation of the normal feedback response.

Borenstein et al. (1983) studied the effects of partially purified atrial and ventricular extracts on renal hemodynamics. Total renal blood flow and cortical blood flow were measured in rats using radioactive microspheres for a control period, then a similar period following the injection of atrial or ventricular extracts. Renal papillary plasma flow was determined in a second group of rats using ^{125}I-labeled albumin before or after extract infusion. Significant decreases in blood pressure were noted following atrial, but not ventricular, extract injection. In addition, significant natriuresis, diuresis, chloruresis and kaluresis were seen in response to atrial, but not ventricular, extracts. Total renal blood flow increased from 7.0 to 9.4 ml/minute/g kidney following atrial extract injection. No significant change was seen following ventricular extracts. Atrial extracts also caused a redistribution of cortical blood flow from outer to inner cortical regions. Renal papillary plasma flow was also increased in rats receiving atrial extract injection when compared to control or ventricular extract-injected rats. The conclusion drawn by

the authors was that a redistribution of blood flow from cortex to medulla occurred in response to atrial extracts and that this could explain part, but not all of the increased excretion of ions and water.

Keeler (1982) studied renal vascular and tubular mechanisms in intact rats following atrial extract injection. Significant responses of natriuresis and diuresis were noted in the absence of accompanying increases in renal plasma flow, GFR or filtration fraction. Furthermore, inhibition of prostaglandin synthesis by aspirin (100 mg/kg) or Indomethacin (10 mg/kg) had no effect on the renal responses to atrial extract. It was concluded that a direct tubular mechanism was involved in the natriuresis and diuresis that was independent of changes in renal hemodynamics and did not require the synthesis of a vasodilator prostaglandin.

Baines et al. (1983) examined the effects of partially purified atrial extracts on isolated rat kidneys perfused with artificial solutions. In addition, kidneys vasoconstricted with a continuous infusion of Angiotensin II were also investigated. Atrial extracts produced no change in renal vascular resistance in control kidneys, but consistently lowered resistance in kidneys receiving A II infusion. These results of decreased resistance are consistent with those of Camargo et al. (1983) with regard to the vasoconstricted kidneys, but the increase in renal vascular resistance reported by that laboratory in normal kidneys was not noted by these investigators.

Sonnenberg et al. (1981a) studied the diuresis and natriuresis, in response to atrial extract administration or to volume expansion with whole blood, following treatment with probenecid, a blocker of tubular secretion of organic acids. Probenecid (50 mM) significantly reduced,

but did not prevent the natriuretic response to either atrial extract or blood volume expansion. It was suggested by the authors that luminal concentration of the atrial factor via active secretion was required for it to exert its effect on the tubules and that probenecid had blocked this process. Circumstantial evidence for a role of ANF in blood volume regulation was suggested by their observation that blockade of organic acid secretion also diminished the renal diuretic response to hypervolemia.

In a later study, Sonnenberg et al. (1981b), using micropuncture techniques on intact rat kidneys, demonstrated a large increase in fractional excretion of sodium, following atrial extract administration, that was mainly tubular in origin. Their data showed that proximal tubular sodium handling was not changed by the extract. However, in the medullary collecting duct, fractional reabsorption of sodium fell markedly and this fall accounted for 80% of the large rise in sodium excretion. Decreases in systemic blood pressure were noted following atrial, but not ventricular extract injection. Glomerular filtration rate did not change after injection of either extract. The authors favored a tubular rather than vascular mechanism to explain the natriuretic effects of atrial extracts.

The above studies demonstrate a profound effect of ANF on the kidney. This effect occurs independently of extrarenal influences because its effect can be demonstrated in isolated kidneys, but this does not rule out extrarenal modulation of its effects. Both tubular and renovascular mechanisms of action have been suggested and it seems likely that the final response is a combination of both of these effects.

Vascular effects of ANF

In addition to the above mentioned renovascular effects of ANF, it has become apparent that ANF is capable of exerting an effect on the extrarenal vasculature as well. Relaxation of precontracted vascular tissue has been reported and ANF may antagonize the action of vasoconstrictor agents as well. Numerous laboratories have reported this vasorelaxant effect of ANF, however its effects on vascular resistance in vivo are less clear.

Kleinert et al. (1984) examined the effects of boiled atrial supernates and partially purified atrial extracts on the in vitro response of vascular smooth muscle to several different agonists. Aortic tissue rings from white New Zealand rabbits were suspended in aerated Krebs buffer and tension development was measured during cumulative addition of either Angiotensin II or norepinephrine. Following re-equilibration, the response to these agonists was re-evaluated in the presence of differing concentrations of the atrial extracts or supernates. Responses to 15 mM K^+ were also evaluated in the same manner. Significant right hand shifts in the dose-response curves to these agonists were reported in the presence of either boiled or partially purified atrial extracts. The degree of inhibition of the contractile response was shown to be dependent on the concentration of atrial extract present in the bathing solution. The response to 15 mM K^+ was also inhibited in a concentration dependent manner, indicating that the effect of the atrial factor was not confined to antagonism of the receptors to Angiotensin II or norepinephrine, but instead interfered with some other event in the cascade leading to vascular smooth muscle contraction.

Currie et al. (1983) reported potent vasorelaxant activity in atrial extracts semipurified from atrial tissue of rats, pigs and man. Using rabbit aortic strips precontracted with norepinephrine, and isolated chick rectum strips maintained in tone by continuous carbachol infusion, a dose-dependent relaxation of these bioassay tissues was demonstrated in response to fractions from column chromatography of atrial extracts. A large molecular weight fraction (20,000 to 30,000 daltons) was described as Peak I and a lower weight fraction as Peak II (MW<10,000). Diuretic and natriuretic activity was present in both peaks. It was proposed that a factor, released from the atria in response to an increase in extracellular fluid volume, stimulates rapid loss of fluid through the urine, and dilation of the peripheral vasculature.

Scivoletto and Carvalho (1984) studied the vascular relaxation effects of atrial extracts on norepinephrine-induced contractions in the presence and absence of endothelial cells. Two pieces of thoracic aorta were removed from the same rat. One was left intact to serve as a control, while the other was gently scraped free of endothelial cells. Following equilibration at resting baseline tension, the rings were both precontracted with 100 nM norepinephrine and the relaxant effects of atrial extract, papaverine and acetylcholine were evaluated. Atrial extracts, but not ventricular, evoked concentration-dependent relaxation of both preparations. Papaverine produced similar results. Acetylcholine, as expected, caused relaxation only in rings with the endothelium intact. The authors concluded that atrial extracts, like papaverine, caused endothelium-independent relaxation of vascular smooth muscle in contrast to acetylcholine, bradykinin or histamine which

require an intact endothelial cell layer.

Garcia et al. (1984) investigated the effects of partially purified rat atrial natriuretic factor on a variety of vascular preparations exposed to pharmacologic agents. ANF produced a dose-related relaxation in rabbit aortic and renal arterial strips precontracted with an ED_{50} dose of norepinephrine. The relaxation began immediately and was complete in four minutes for the renal artery and in eight minutes for the aorta. No relaxation was seen in rabbit mesenteric arterial strips. Similar results were obtained using Angiotensin II as a agonist, while no effect was noted during K^{+}-induced contractions. The relaxation of norepinephrine or A II-induced contractions were not modified by the addition of propranolol, atropine, indomethacin, methylene blue or the use of Ca^{++}-free media. Experiments using the isolated rat mesenteric vascular preparation gave results similar to those for rabbit mesenteric strips, where ANF infusion produced neither changes in baseline resistance or refractoriness to norepinephrine. When ANF was bolus injected during continuous norepinephrine infusion, no significant changes in perfusion pressure were observed. The isolated rat kidney, perfused with artificial solutions, was also tested. Bolus injection of ANF caused an immediate fall of 33 mmHg in perfusion pressure which lasted 18 minutes. The authors concluded that a direct relaxant and vasodilator effect on several vascular preparations was attributable to ANF. This action was not mediated through competition at alpha receptors since it relaxes tissue precontracted with A II as well. Beta adrenergic or cholinergic receptors are apparently not involved, nor is there a requirement for extracellular Ca^{++}. This absence of Ca^{++} dependency is in apparent

contrast to the results of Camargo et al. (1984), where an absolute requirement for Ca^{++} entry was demonstrated in the renal response to ANF. Perhaps the renal and vascular responses differ in this respect.

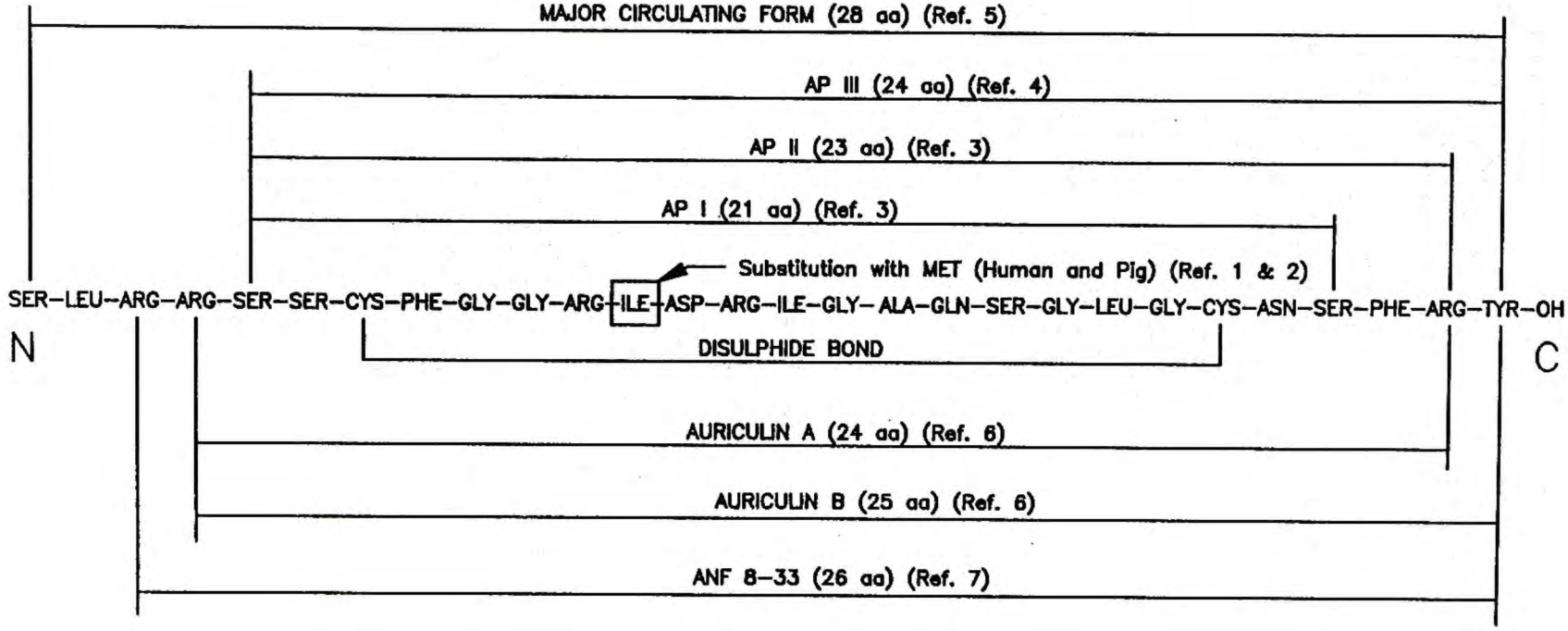
The above studies suggest that ANF may play a role in modulating vascular tone by nature of its effects on large arteries. However, a similar effect on small resistance vessels has not been demonstrated. The antagonism of vasoconstrictor agents may be of greater physiological importance than the direct vasorelaxant effects reported above.

Primary structure and biological actions of ANF

Investigation of the chemical nature of the natriuretic activity in atrial extracts lead to the isolation and characterization of a family of peptides collectively called ANF. Some of these peptides and related fragments have been synthesized, are available commercially and are being actively investigated. It should be made clear at this point that these fragments have been isolated and characterized from atrial tissue homogenates and may not reflect the active form in the circulation.

Currie et al. (1984) reported purification of two peptides with natriuretic activity from rat atria. The first of these two peptides (called Atriopeptin I, or AP I) consists of 21 amino acids and relaxed intestinal, but not vascular, smooth muscle (Currie et al., 1984). Its amino acid sequence from amino (N) terminal to carboxyl (C) terminal is shown, along with other forms, in Figure 1.

FIGURE 1. Schematic representation of Atrial Natriuretic Factor (ANF). The commonly reported fragments of the molecule are depicted, along with the references where their structures were first described.



REFERENCES:

1. Kangawa and Matsuo, 1984
2. Forssman et al., 1984
3. Currie et al., 1984
4. Geller et al., 1984
5. Schwartz et al., 1985
6. Atlas et al., 1984
7. Seidah et al., 1984

The second peptide, Atriopeptin II (AP II) had the same sequence with a PHE-ARG residue on the C-terminus (23 amino acids), and relaxed vascular as well as gastrointestinal smooth muscle. It was roughly four times as potent in causing natriuresis as AP I (Currie et al., 1984).

Geller et al. (1984) isolated six biologically active peptides from rat atria by ion exchange chromatography of two natriuretic peaks obtained by gel filtration. The first peak contained Atriopeptin I and three homologs. The second peak contained AP II and another peptide with the same sequence as Atriopeptin II, with the addition of a C-terminal TYR residue (termed Atriopeptin III). All six peptides contained identical 17-membered rings formed by an internal disulphide bond between the two Cysteine residues. A common, high molecular weight precursor was suggested by the investigators.

Atlas et al. (1984) reported purification, sequencing and synthesis of 24 and 25 amino acid peptides with natriuretic and vasorelaxant activity. The 24 amino acid form (called Auriculin A) shares the same amino acid sequence with AP II with the addition of a N-terminal ARG residue. Auriculin B, the 25 amino acid form is equivalent to AP III, but has the same N-terminal ARG residue as Auriculin A. Synthetic forms of the peptides had vasorelaxant activity that paralleled the purified native forms.

Seidah et al. (1984) isolated four peptides, the longest containing 33 amino acids. The other three were amino-terminally truncated versions of the 33 amino acid form, containing 32, 31 and 26 amino acids. The 26 amino acid form, termed ANF 8-33, was shown to have vasorelaxant potency at least as great as the longer chain forms and was synthesized. The synthetic form was equivalent in vasorelaxant activity

to the 31 amino acid native peptide. ANF 8-33 has an amino acid sequence the same as Auriculin B with the addition of another ARG residue to the N-terminus.

Kangawa and Matsuo (1984) purified and sequenced an atrial peptide from human cadavers within ten hours postmortem. Termed alpha-human Atrial Natriuretic Peptide (alpha-hANP), it contained 28 amino acids and a sequence similar to ANF 8-33 with two differences: the addition of SER-LEU to the N-terminus and the substitution of MET for ILE on the 17th position of ANF 8-33. The peptide possessed potent natriuretic, diuretic and vasorelaxant properties similar to those shown by crude atrial extracts.

Kangawa et al. (1984) isolated, purified and sequenced a 48 amino acid polypeptide termed beta-rat Atrial Natriuretic Polypeptide (beta-rANP). This compound has the same C-terminal 26 amino acid sequence of ANF 8-33 with a 22 amino acid sequence on the N-terminus. The natriuretic activity of this peptide was roughly equivalent to that of alpha-hANP.

de Bold and Flynn (1983) reported purification of a 49 amino acid polypeptide, from rat atrial tissue, with potent diuretic and natriuretic properties. The peptide was termed Cardionatrin I, and complete sequence was not presented. Amino acid analysis demonstrated only one CYS residue and the peptide is therefore different in composition from the above mentioned peptides containing two CYS residues. Later the same authors (Flynn et al.,1983) presented the sequence of a 28 amino acid peptide, with a sequence identical to ANF 8-33 with the addition of SER-LEU to the N-terminus. The biological activity of this peptide was reported as equivalent to that of

Cardionatrin I.

Misono et al. (1984) reported isolating four peptides, with natriuretic and smooth muscle relaxant activity, containing 35, 31, 30 and 25 amino acids. The predominant form was that of the 31 amino acid form and its sequence corresponded to that of ANF 8-33 plus five amino acids (GLY-PRO-ARG-SER-LEU) at the N-terminus. Cleavage by staphylococcal protease at the ASP residue, between the two CYS residues, attenuated the biological activity indicating that the disulphide bond between the CYS residues was essential for this activity.

Forssman et al. (1984) isolated and characterized a 126 amino acid polypeptide (called Cardiodilatin 126) from porcine right atrium which possessed vasorelaxant properties when tested in bioassay on smooth muscle strips from aorta, renal and inferior mesenteric arteries from rabbits. Natriuretic and diuretic effects were demonstrated by bolus injection in conscious dogs. Smaller peptides were found during the isolation procedure, one containing 88 amino acids (Cardiodilatin 88) which possessed the same biological activity. Sequencing of Cardiodilatin 126 demonstrated that the peptides reported in the above studies (AP I, II, and III, Auriculin A and B) are C-terminal fragments of this larger peptide, with the MET for ILE substitution mentioned earlier. In the human, alpha-hANP is identical to the 99-126 segment of Cardiodilatin 126.

The above study demonstrates that the active atrial peptides probably exist in a higher molecular weight precursor form. Trippodo et al. (1984) demonstrated both high (10 -40K) and low (10K) molecular weight fractions and that their relative proportions varied according to

the extraction procedure used. Extraction in 1.0 N acetic acid yielded predominantly the high molecular weight form, while the use of 0.1 N acetic acid yielded extracts with a predominance of the low molecular weight form. When the high molecular weight form was partially purified and eluted with 0.1 N acetic acid, it remained intact, unless exposed to crude atrial extracts in 0.1 N acetic acid where a conversion to the low molecular weight form occurred. When extracted in boiling 0.1 N acetic acid, this conversion was prevented. It was concluded that the high molecular weight form predominates in the atria and that extraction in 1.0 N acetic acid results in this form being extracted intact. A heat labile activity that converts the high molecular weight form to the low form was postulated, and that in 1.0 N acetic acid or boiling 0.1 N acid, its activity is suppressed.

Raine et al. (1984) examined the effects of high and low molecular weight fractions, purified from rat atria, on isolated, perfused rat kidneys. Both fractions were found to be active in the kidney where there was a small increase in GFR followed by natriuresis. The onset of natriuresis was faster following the low molecular weight form as compared to the high form. It was suggested by the authors that intrarenal conversion of the high molecular weight form into the low form was involved.

Currie et al. (1984b) demonstrated increased smooth muscle relaxant activity of high molecular weight fractions following mild proteolytic treatment with Trypsin, urinary or submaxillary Kallikrein. Conversion to the low molecular weight form accompanied the enhanced activity of the atrial extracts. Treatment with Aprotinin prevented this conversion and enhancement of activity, while Soybean Trypsin

Inhibitor was without effect. It was suggested that the low molecular weight form was more active and that mild proteolysis of the high molecular weight form activates the vasorelaxant activity by conversion of the lower molecular weight forms.

Garcia et al. (1985) studied the effects of chain length modifications on the vasorelaxant effect of ANF 8-33 on the thoracic aorta of rabbits. Removal of individual amino acids was achieved by N-terminal chemical cleavage or C-terminal enzymatic digestion. Removal of the four amino acid residues of the N-terminus (ARG-ARG-SER-SER) had little effect on the vasorelaxant activity. In contrast, at the C-terminus, removal of the C-terminal TYR residue caused a large drop in vasorelaxant activity. Subsequent removal of ARG or PHE-ARG caused a progressive decrease in activity, and when the last five C-terminal residues (ASN-SER-PHE-ARG-TYR) were removed, activity was almost completely lost. This study demonstrates the importance of the C-terminal amino acids for biological activity. The PHE-ARG residues are necessary for vasorelaxant activity and the addition of TYR apparently enhances this action.

Wakitani et al. (1985) demonstrated the necessity of the PHE-ARG residue on the C-terminal for renal vasodilator activity in isolated, perfused rat kidneys. The purified high molecular weight fraction was ineffective relative to AP II and III, and reduced renal vascular resistance only after proteolytic cleavage. This finding is in apparent contrast to those of Forssman et al. (1984) where the high molecular weight form was shown to have this activity. Perhaps in that study the high molecular weight form was allowed to be degraded by proteases in the extract to the more active low molecular weight form and this

degradation was not noticed. Species differences could also have contributed to this contrasting finding.

Windquist et al. (1984) demonstrated the vasorelaxant effects of ANF 8-33 on rabbit facial vein and thoracic aorta. The facial vein, with intrinsic tone similar to small arteries and arterioles, was most sensitive to the vasorelaxant effects of the synthetic peptide. A similar vasodilator profile was reported for sodium nitroprusside and a common mechanism was suggested by the authors.

Seymour et al. (1985) measured the renal and hemodynamic effects of ANF 8-33 on anesthetized dogs. Natriuresis and diuresis were reported in the absence of significant elevation of GFR. Mean blood pressure was decreased without reflex tachycardia. Cyclic GMP levels were reported increased, but were shown to be extrarenal in origin. The authors concluded that a direct renal action was responsible, for the natriuresis and diuresis, that did not depend on changes in renal hemodynamics or cGMP levels.

Yukimura et al. (1984) studied the renal effects of alpha-hANP infusion in anesthetized dogs. While large doses (1 microgram/minute) resulted in increases in GFR and renal blood flow, blood pressure was not affected. Lower doses (0.2 micrograms/minute) still caused natriuresis and diuresis, but did not affect GFR or renal blood flow. These results of natriuresis without elevated GFR are similar to those of Briggs et al. (1982) mentioned previously where atrial extracts rather than synthetic forms of the peptide were infused. A tubular mechanism of action was proposed by the authors to explain the responses at low doses, while at higher doses, renal hemodynamics and GFR were also affected, which adds to the observed natriuresis.

In addition to its effects on the kidneys and vascular tissue, ANF also has effects on hormonal systems which are involved in regulating fluid volume and vascular tone. Maack et al. (1984) measured renal function, blood pressure, renin secretion rate, plasma renin activity and plasma aldosterone levels in dogs following intravenous infusion of Auriculin A. This purified 24 amino acid peptide, obtained from rat atria, lowered blood pressure, increased GFR and caused natriuresis and diuresis in these dogs. These responses returned to control levels following cessation of the Auriculin A infusion. Renin secretion, plasma renin activity and plasma aldosterone levels were all significantly decreased following Auriculin A administration.

Together, the above studies suggest that the endogenous circulating active form of atrial natriuretic factor is still not known. The activity of atrial extracts may be due to the combined effects of many peptides that differ only slightly in amino acid sequence and may come from a common precursor. The differences in the primary structures reported may be due to the different techniques used in isolation, purification and sequencing. The reported structures share similar vasorelaxant activity as long as the PHE-ARG on the C-terminal is present. The addition of TYR seems to increase the activity. The chain length at the N-terminal is apparently not involved in this activity. The human and porcine forms of ANF bear a striking similarity to those from the rat, differing in only one amino acid. Selective proteolytic cleavage of a high molecular weight precursor may be involved in the biological actions of these peptides.

Cellular mechanisms involved in ANF action and release

The mechanism of action of ANF on the kidneys and cardiovascular system still remains unknown. Recent studies have implicated cyclic nucleotide metabolism with the action of ANF, possibly in a second messenger capacity. Another study suggests a role of ANF in modulating Ca^{++} fluxes.

Hamet et al. (1984) measured cyclic GMP levels in intact rats, primary culture renal cortical cells and kidney slices following treatment with purified atrial extracts or synthetic ANF 8-33. Atrial extract injection in intact rats resulted in a 28-fold increase in urinary cGMP excretion. A decrease in cAMP levels followed. Atrial extract treatment resulted in a three-fold increase in cGMP levels in kidney slices, a 2.5-fold increase in cultured cortical cells and a six-fold increase in the cultured cell supernates.

Waldman et al. (1984) reported activation of particulate guanylate cyclase, and increased levels of cGMP in crude membrane fractions, obtained from kidney homogenates, following incubation in synthetic ANF. No effect on adenylate cyclase was observed.

Schenk et al. (1985) demonstrated specific, saturable, competitive binding of ^{125}I-labeled rat pro-ANP to cultured bovine smooth muscle and endothelial cells and concurrent stimulation of cGMP levels. A similar order of potency was found in binding and stimulation of cGMP, where rat pro-ANP (102-126) equaled that of human pro-ANP (102-126) and both showed greater potency than reduced human pro-ANP.

While the effect of ANF in stimulating cGMP levels is apparent, whether cGMP plays a role in mediating its effects is still obscure.

Studies by Katsuki and Murad (1977) demonstrated that increases in cGMP levels were associated with both contraction and relaxation of bovine tracheal smooth muscle and that the relative changes in cGMP and cAMP levels were dependent on the agonist used. Carbachol, acetylcholine and histamine caused contraction and increased cGMP. Guanylate cyclase activators such as sodium azide, hydroxylamine, sodium nitrite, nitroglycerin and sodium nitroprusside all increased cGMP levels but caused relaxation. Without further knowledge of the role of cGMP in smooth muscle contractility, any explanation for the vascular effects of ANF that involves cGMP would be speculative.

Stimulation of cGMP levels have been associated with increases in electric potential difference and short circuit current in rabbit ileal mucosa (Field et al., 1978). If a similar effect occurs in the kidney, stimulation of cGMP by ANF could, in part, explain some of the tubular effects seen. Atrial supernates, however, do not have observable effects on short circuit current in the toad bladder (Pamnani et al., 1984).

While the mechanism is not clear, from the above studies it can be seen that cGMP is involved in smooth muscle contractility and membrane ion transport. These are in common with the most apparent effects of ANF, those of modulating vascular tone and stimulating sodium excretion. Still, the association can only be considered coincidental at this time.

Taylor and Meisheri (1985) studied the effects of AP II on unidirectional Ca^{++} fluxes in rabbit aortic rings. AP II produced 97% relaxation of norepinephrine-induced contractions while only relaxing K^{+}-induced contractions by 17.5%. Using 90 second pulse labeling with

$^{45}Ca^{++}$, it was demonstrated that norepinephrine-induced Ca^{++} influx was inhibited 60% by AP II while K^{+}-induced Ca^{++} influx was unaffected. Treatment with 1.0 micromolar D-600 inhibited the K^{+}-induced Ca^{++} influx. The authors concluded that AP II had a more selective inhibitory effect on Receptor-Operated Ca^{++} channels than on Potential-Operated Ca^{++} channels.

Sonnenberg and Veress (1984) reported release of ANF from incubated atrial tissue following exposure to acetylcholine. It was demonstrated that muscarinic cholinergic stimulation with acetylcholine caused release of ANF coupled with a corresponding decrease in atrial ANF content. Release could not be demonstrated following treatment with ouabain, isoproterenol or high extracellular K^{+}. The release in response to acetylcholine was shown not to be due to cell disruption because it could be blocked by atropine. Adrenaline, too, caused a specific release that could be antagonized with phentolamine. Argenine vasopressin, but not deamino-8-D-argenine vasopressin, also caused release. The authors suggested that a common feature of vasopressin, adrenaline and acetylcholine action was the activation of the phosphoinositol pathway resulting in inositol triphosphate release, and that this inositol triphosphate was a second messenger of stimulation-secretion coupling in atrial cells. Inositol triphosphate levels were not determined however.

Role of ANF in body fluid volume regulation

Several lines of evidence suggest a role for ANF in the regulation of body fluid volume. Atrial granularity changes with changes in water balance (de Bold, 1979), immunoreactive ANF decreases with water deprivation and increases with saline loading (Gutkowska et al., 1985), and atrial levels of ANF have been shown to be increased in reduced renal mass hypertensive rats drinking 1% saline (Pamnani et al., 1984) a model where extracellular fluid volume has been shown to be elevated (Huot et al., 1983). Recently, several studies have demonstrated release of ANF in response to stimulation of the atria by stretch or by acute increases in extracellular fluid volume, a method known to elicit natriuretic and diuretic responses (Linden, 1979).

Veress and Sonnenberg (1984) measured the renal excretory response to acute expansion of blood volume with isotonic Ringers with albumin. Removal of the right atrial appendage was performed in experimental rats by placing a loop ligature around the atrial appendage. As compared to sham-operated control rats, the resulting natriuresis and diuresis in experimental rats was reduced 50% by the procedure. This difference in sodium and water excretion was not altered by prior bilateral vagotomy. In addition, the administration of homologous rat atrial natriuretic factor (the equivalent of one atria), or furosemide, resulted in identical renal responses in both the experimental and control groups. The authors concluded that acute hypervolemia was associated with the release of ANF into the bloodstream and that removal of the atrial appendage reduces the amount available for release. Their results with vagotomized animals suggest that an

afferent neural mechanism is not necessary for the renal response to occur.

Dietz (1984) demonstrated a possible role for ANF in control of blood volume using an isolated rat heart-lung preparation. Increasing the central venous pressure by raising the perfusate resevoir caused atrial distention. The perfusate was collected and used in bioassay for natriuretic activity. Perfusates collected during periods of atrial distention caused a larger natriuresis in bioassay rats than perfusates collected during periods of low central venous pressure. Dietz concluded that atrial distention caused the release of the natriuretic activity from the atria.

Lang et al. (1985) studied the release of ANF-like material from rat atria in vitro using an isolated, perfused rat heart preparation. Increasing the perfusion pressure resulted in increased right atrial pressure. When right atrial pressure was increased only 1mmHg, the rate of ANF secretion into the perfusate doubled. Increasing the pressure 5mmHg resulted in a two to three fold increase in perfusate ANF levels. In addition, HPLC analysis of the released ANF demonstrated that it consisted of the low molecular weight form and eluted with a retention time between that of AP II and AP III standards. Little of the high molecular weight form was found to be present in the perfusate.

Goetz et al. (1985) compared the natriuretic effects of left atrial distention with those of atriopeptin (II or III) infusion. Renal effects of the two manipulations were similar, and it was suggested that the renal natriuretic response to atrial distention may be mediated largely by the release of natriuretic peptides from the atria.

Ledsome et al. (1985) measured immunoreactive ANF levels in

plasma following atrial distention by mitral valve obstruction. Increased levels of ANF were reported following distention, and this response was not attenuated by bilateral vagotomy. Electrical stimulation of the right ansa subclavia raised heart rate but did not affect ANF levels in the plasma. The authors concluded that atrial distention caused the release of ANF directly, not through a reflex mechanism or as the result of increased sympathetic efferent nerve activity at the heart.

Courneya et al. (1985) extended these studies and followed the time course of the increased plasma ANF levels. Following mitral obstruction, plasma ANF increased within two minutes, and the levels had reached a maximum by five minutes. ANF levels returned to control following removal of the mitral valve obstruction.

Thrasher et al. (1985) reported increased immunoreactive ANF in plasma of dogs following increased left atrial pressure. Changes of 2.4 to 3.5 times as much ANF were demonstrated in plasma after left atrial pressure was maintained elevated for 60 minutes.

The above studies suggest that a feedback mechanism exists where increases in blood volume cause increased stretch of the atria which releases ANF that acts, in turn, on the kidneys to increase salt and water excretion and bring the blood volume back towards normal. Other studies have suggested a role for ANF in situations where abnormal control of body fluid volume is apparent.

Chimoskey et al. (1984) measured atrial ANF levels, by natriuresis bioassay in rats, of BIO 14.6 hamsters and compared them to atrial levels of normal age-matched control hamsters. BIO 14.6 hamsters are genetically prone to cardiomyopathy which results in decreased

cardiac mechanical performance, and develop congestive heart failure and edema at about 200 days of age. Assay of atrial extracts demonstrated that the BIO 14.6 hamsters were deficient in ANF, having approximately one half the activity of extracts from normal hamsters. These results suggest that decreased levels of ANF may be partly responsible for the fluid retention observed in these animals.

The above studies suggest a role for ANF in the control of body fluid volumes independent of the central nervous system. Recent studies suggest that the CNS is also involved with ANF and its role in fluid volume regulation. Jacobowitz et al. (1985) demonstrated that ANF-containing neurons are present in the brain in regions believed to be involved in volume regulation as well. The authors found immunoreactive ANF-positive cell bodies in the organum vasculosum of the lamina terminalis and in several hypothalamic nuclei including the periventricular, arcuate and ventral premammillary nuclei. Nerve fibers containing ANF were generally observed in the vicinity of the cell bodies.

Similar findings were reported by Saper et al. (1985), where atriopeptin-immunoreactive neurons were found in the medial and periventricular preoptic nuclei. It was suggested that blood-borne atriopeptins may have access to these nuclei through regions where the blood-brain barrier is lacking, such as the organum vasculosum of the lamina terminalis, and by this mechanism can be involved in central nervous system regulation of blood and extracellular fluid volume.

Role of ANF in control of blood pressure

Since extracellular fluid volume regulation is intimately related to blood pressure regulation, there is increasing evidence for a role of ANF in the control of blood pressure as well. Rapid decreases in arterial blood pressure in response to ANF administration have been demonstrated in numerous previously mentioned studies (Borenstein et al., 1983; Sonnenberg et al., 1982; de Bold et al., 1981; and Maack et al., 1984). Several mechanisms seem to be involved in this response. A direct vasorelaxant effect on arterial preparations has been mentioned, as well as antagonism of several vasoconstrictors.

Osol and Halpern (1985) studied the effects of ANF on small (approximately 200 micron diameter) cerebral and mesenteric arteries. In contrast to the effects of ANF on vascular smooth muscle preparations from large arteries, addition of ANF did not cause any transient or sustained changes in diameter in cerebral arteries at resting tone, or mesenteric arteries precontracted with 30 mM K^+, serotonin, $PGF_{2\text{-alpha}}$ or transmural electrical stimulation. The authors concluded that the hypotensive effects of ANF may not be mediated by decreases in total peripheral resistance, at least not in the brain or gut.

Recently, other investigators have shown that the decrease in blood pressure may not be entirely due to decreased total peripheral resistance, and that cardiac output may be decreased as well. Kleinert et al. (1985) measured the cardiovascular effects of infusion of synthetic 24 amino acid ANF in normal dogs. Mean arterial pressure dropped while total peripheral resistance increased and remained elevated for one hour after infusion. Heart rate remained unchanged during, and after the infusion. Cardiac output decreased during the

infusion and remained depressed for one hour during the recovery period.

Natsume et al. (1985) reported the effects of Atriopeptin III on systemic hemodynamics in anesthetized rats. Mean arterial pressure decreased 7% and cardiac output decreased 14%. Total peripheral resistance increased 10%. Cardiac performance, blood volume and mean circulatory filling pressure were not altered following injection. The authors conclusions were that mean arterial pressure was reduced partly by decreased cardiac output, and that the decrease in cardiac output was not the result of myocardial depression, venous dilitation or decreased blood volume.

Brandt et al. (1985) reported decreases in arterial pressure and cardiac output, in conscious sheep, in response to infusion of 23 amino acid ANP. Total peripheral resistance was slightly elevated and heart rate, in contrast to other studies where reflex tachycardia was absent, was increased from 68 to 80 beats per minute.

An interesting observation during the hypotensive episode following ANF administration is the absence of reflex tachycardia (Seymour et al., 1985; Metzler et al., 1985; and Tang et al., 1985). This could be explained by the study of Thoren et al. (1985) where the lowering of arterial pressure in response to AP III was attributed to several mechanisms. They measured changes in sympathetic renal nerve activity following AP III in the intact state and after sinoaortic denervation with and without bilateral vagotomy. In intact rats, AP III decreased arterial pressure without reflex increases in sympathetic nerve activity. In rats with sinoaortic denervation, the fall in blood pressure was accompanied by decreased nerve activity. Bilateral vagotomy attenuated the decrease in sympathetic nerve activity. The

authors concluded that AP III activates inhibitory cardiac vagal afferents and this action inhibits the expected increases in renal sympathetic nerve activity during the hypotension. This decreased nerve activity could be responsible for part of the renal vasodilation and natriuresis observed during AP III infusion. The absence of reflex tachycardia could then be due to a generalized decrease in sympathetic nerve activity.

The above studies demonstrate a possible role for ANF in regulating blood pressure on a short-term basis. However, several observed effects of ANF could be involved in blood pressure control over a longer time period. The effects of ANF on volume regulation and the role of fluid volume in blood pressure control have been discussed. In addition, ANF has effects on a hormonal system intimately involved in the short and long term control of blood pressure, that of the renin - angiotensin - aldosterone system.

Burnett et al. (1984) studied renal function and renin release from anesthetized dogs following intrarenal infusion of synthetic ANF. A transient increase in renal blood flow for approximately three minutes was followed by decreased renal blood flow and an increase in GFR. Renin secretion was markedly decreased from 295.5 to 17.2 nanograms per minute during the infusion, despite a small decrease in blood pressure, and returned to control levels after infusion was stopped.

Vari et al. (1985) evaluated the influence of synthetic ANF on plasma renin activity and adrenal aldosterone secretion rate in normal rats. Mean arterial pressure and adrenal plasma flow were unaltered, while plasma renin activity and aldosterone secretion both decreased significantly.

Atarashi et al. (1984) measured basal, ACTH and Angiotensin II - stimulated aldosterone release from rat zona glomerulosa cell suspensions. Treatment with atrial extract decreased basal release and partially inhibited ACTH or A II - stimulated release of aldosterone. Treatment with trypsin abolished this activity of the atrial extracts, suggesting that ANF was involved.

Chartier et al. (1984) examined the actions of natural ANF 8-33, ANF 1-73 and synthetic, oxidized ANF 48-73 on aldosterone secretion by isolated rat zona glomerulosa cells. ANF 8-33 and the 1-73 form both inhibited the Angiotensin II - stimulated secretion of aldosterone, while the oxidized 48-73 form was without effect. The 8-33 and 1-73 forms of ANF also inhibited ACTH and K^+-stimulated secretion of aldosterone.

Goodfriend et al. (1984) studied the inhibition of basal and Angiotensin II - stimulated aldosterone release, from adrenal glomerulosa cell suspensions, by the 24 amino acid peptide Auriculin A. The greatest inhibitory influence was on basal levels. Inhibition of Angiotensin II - stimulated secretion by Auriculin A could be overcome by increasing the A II concentration. The inhibition of aldosterone secretion apparently occurred early in the pathway for aldosteronogenesis, as it was shown that Auriculin A inhibited conversion of cholesterol to pregnenolone.

The above studies demonstrate that ANF can affect a hormonal system known to be involved in blood pressure regulation. A role for ANF has also been suggested in several forms of experimental hypertension where differences in ANF levels between hypertensive animals and their controls have been demonstrated.

Sonnenberg et al. (1983) measured atrial levels of ANF in SHR and WKY rats by diuresis bioassay of atrial extracts. Dose response curves were obtained using atrial extracts from WKY rats to establish the dose producing a maximal response in normal assay rats. SHR and WKY atrial extracts were assayed using a quantity of extract less than this to rule out the possibility of a maximal dose being given to both of the assay rats thereby obscuring any possible differences in ANF content between the two extracts. Atria from SHR were found to contain less ANF than equal tissue weights of atria from WKY rats. The authors concluded that chronic release of ANF from SHR atria had depleted the atrial stores and that increased blood levels of ANF may be involved in their hypertension. Blood or plasma levels of ANF were, however, not determined.

Xie et al. (1985) measured plasma levels of ANF in SHR and WKY rats by radioimmunoassay. Immunoreactive atriopeptin levels were found to be seven times higher in plasma of SHR relative to WKY rats.

Awazu et al. (1985) measured hemodynamic responses of SHR and WKY rats to the intravenous infusion of ANF 8-33. The peptide decreased blood pressure significantly in SHR but not in WKY rats. GFR was increased in a similar manner in both strains.

Irizawa et al. (1985) evaluated the responsiveness of hypertensive rats, treated with DOCA and drinking saline, to the effects of ANF from normal rats. DOCA rats showed less natriuresis than similarly injected control rats. Whether this difference in responses was due to altered metabolism of ANF or decreased responsiveness to its effects was not determined. DOCA-treated rats have higher atrial levels of ANF (de Bold, 1979) and this may be compensatory to the decreased

responsiveness or altered ANF metabolism suggested in this study.

Chinn and Hartle (1985) demonstrated differences in responsiveness to ANF between perinephretic hypertensive rats and one-kidney control rats. While i.v. injection of rat ANF produced diuresis and natriuresis in control rats, there was virtually no effect in the hypertensive rats. The authors suggested that the lack of responsiveness was due to impairment of extracellular fluid volume regulation that could contribute to the development of hypertension.

The above studies suggest that altered levels of ANF may be involved in the development or maintenance of hypertension. The question remains as to whether the alterations in ANF levels and sensitivity are responsible for part of the pathogenesis of hypertension, or secondary to it. One genetic model of hypertension, that of the Dahl Strain of Salt-Sensitive rats, may provide some clues. It is one model where altered renal handling of salt exists prior to any hypertension, and does not require the use of invasive procedures or pharmacologic manipulations in order to produce hypertension.

THE DAHL MODEL OF SALT-SENSITIVE AND SALT-RESISTANT RATS

While the aforementioned studies on salt intake clearly show a relationship between dietary sodium chloride intake and the mean blood pressure for a group of animals or humans, the individual blood pressure responses within the group vary greatly. It has been suggested that genetic factors play a role in sodium chloride-induced hypertension, where, in a given population source, certain individuals of this group

are more susceptible than others to increased dietary sodium chloride intake.

This concept was utilized by Dahl et al. (1962) to obtain strains of rats differing only in their susceptibility to sodium chloride-induced hypertension. Large populations of Sprague Dawley rats were screened, by the levels of their arterial blood pressure, for their response to high dietary sodium chloride. Rats showing increases in blood pressure were separated from those showing no response, and by close inbreeding (brother-sister) within the two groups, they produced two strains of rats: a Salt-Resistant (R) strain and Salt-Sensitive (S) strain. S rats become hypertensive when fed a high salt (8.0%) diet, while R rats remain normotensive on this salt intake. S rats fed a low salt (0.4%) diet remain normotensive but still show an elevation of the group average blood pressure of approximately 10 mmHg over that of similarly fed R rats. The mechanism of this hypertension, as with most experimental models, remains unknown.

This model in many respects resembles human essential hypertension where a genetic predisposition is also indicated. These rats also respond to diuretic treatment, and the use of antagonists of the sympathetic nervous system, by a lowering of blood pressure as is seen in many essential hypertensives. The rats differ only in their response to salt, so surgical manipulation or the addition of vasoactive substances in physiological or non-physiological amounts is not required either. The Dahl strain of Salt Sensitive and Salt Resistant rats is the model I have chosen to study for my thesis.

Work done by other investigators in the Dahl model of hypertension

Dahl et al. (1968) studied the effects of increased dietary sodium chloride intake on blood pressure in salt sensitive 'S' rats with respect to concentration, the effects of transient increases in sodium chloride intake, and the age at which increased intake began. Compared to rats eating 0.4% sodium chloride diets, rats on 1.0, 2.0, 4.0 and 8.0% sodium chloride diets all showed significant increases in blood pressure, in almost linear fashion, with a 15 mmHg rise in pressure for each doubling of salt intake. In addition, animals fed 8.0% NaCl diets for six weeks after weaning had higher blood pressure than rats on the same diet for only two weeks after weaning. This difference was still evident 12 months later even though both groups resumed eating the low salt diet for the entire year after the two or six week period of 8.0% NaCl intake. Furthermore,the rats fed 8.0% NaCl for only six weeks were not different in blood pressure from rats fed the same diet for the entire year. The age at which the high salt intake begins also determines the severity of the resultant hypertension. Blood pressure was significantly higher in rats started on 8.0% NaCl at weaning than in those groups starting three or six months later. There was, however, no difference in blood pressure between those starting at three or six months.

Ganguli et al. (1979) measured hemodynamic parameters of cardiac output, total peripheral resistance and systemic blood pressure during salt feeding in Dahl rats. Feeding of 8.0% sodium chloride to S rats caused simultaneous increases in cardiac output and peripheral resistance and therefore blood pressure. After three days on the high

salt diets, S rats showed increases of 10% in both cardiac output and resistance and a resultant 20% increase in blood pressure. R rats showed an increase of 18% in cardiac output in response to high salt, but their peripheral resistance fell 14% below control and therefore blood pressure was not changed significantly. Following seven days on the high salt regimen, cardiac output had returned to near normal in S rats while peripheral resistance and blood pressure remained significantly elevated as compared to S rats consuming low salt (0.3%) for the same time period. The authors speculated that the baroreceptors in S rats were subject to resetting to a higher operating range when the rats were exposed to high salt intake. Then, when the cardiac output and blood pressure rise, the baroreceptors fail to respond with peripheral vasodilation, and blood pressure remains elevated. When R rats are exposed to the same high salt diet, the baroreceptors allow a fall in resistance and therefore maintain normal blood pressure.

This dichotomy in hemodynamic responses to increased dietary sodium chloride intake between S and R rats bears a close resemblance to a study on human borderline hypertensives. Mark et al. (1975) evaluated the effects of high and low sodium intake on borderline hypertensive subjects and compared the responses to those of normal volunteers. Following 10 days of high salt intake (410 mEq/24 hours), decreased forearm blood flow, and increased forearm vascular resistance and systemic blood pressure were noted in the borderline hypertensives. High sodium intake also augmented the vasoconstrictor response in the forearm following lower body negative pressure, a stimulus for neurogenic vasoconstriction. Responses to elevated salt intake in normotensives were in contrast with those of borderline hypertensives.

The same excess salt intake in normotensives resulted in increased forearm blood flow, decreased resistance and failed to significantly increase systemic arterial pressure. Dahl S and R rats showed responses similar to the borderline hypertensives and normotensives respectively. S rats showed increases in resistance in response to increased salt intake, similar to the borderline hypertensive human subjects. R rats and normotensive humans both show vasodilation in response to elevated salt intake.

Another similarity to human essential hypertension in Dahl S and R rats exists in their response to diuretics. Tobian et al. (1979) studied the effects of pretreatment with methyclothiazide (0.01% w/v) on the response of Dahl S rats to high salt intake. R rats, whether receiving methyclothiazide or not, showed no increase in blood pressure. S rats not receiving the diuretic showed the normal response of an increase in blood pressure, while those receiving the diuretic showed no significant rise in systemic arterial pressure. In a second series of experiments by Tobian et al. (1979), normal Sprague Dawley rat kidneys were isolated and perfused with blood from either S or R rat donors that had been maintained on low (0.3%) salt diets. After an initial control period, the donor rat was volume expanded with a mixture of 66% blood and 33% Ringers solution at a rate of 5% of body weight per hour in order to stimulate the appearance of any natriuretic hormonal agents in the blood of the donor rat. Normal kidneys perfused with blood from S rats showed almost 50% less sodium excretion than kidneys perfused with blood from R rats. This was interpreted by the authors as evidence for an antinatriuretic factor in S rats while on low salt. Results from the volume expansion experiments showed that both S and R rats responded

equally to the volume expansion by increasing sodium excretion in similar fashion. Further evidence for the source of the postulated "sodium-retaining hormone" was not provided.

Structural alterations do not appear to be involved in the development of hypertension in Dahl S rats. Mueller (1983) studied the hindquarter vasculature of Dahl S and R rats before, during and after the establishment of hypertension in S rats by a high salt diet (8.98% sodium chloride). Perfusion pressure, during perfusion of hindquarters at 3.0 ml/minute/100 g body weight, was determined in isolated hindquarter preparations during maximal dilation and vasoconstriction. Maximal vasodilation was achieved using papaverine (0.04 mg/ml) and confirmed by comparison to resistance of the vascular bed following five minutes of ischemia. Maximal vasoconstrictor responses were achieved by injection of vasopressin (10 IU) and Barium Chloride (150 mg) into the perfusate. Norepinephrine sensitivity was determined by performing dose-response curves in the concentration range of 0.01 to 10.0 micrograms per milliliter. Resistance during maximal vasodilation increased with the length of time on the high salt diet in S, but not R rats. This was interpreted by the author as evidence for vascular structural alterations occurring simultaneously with the development of the hypertension but not preceeding it. The maximal vasoconstrictor response was not different between S and R rats when on high, salt and it was suggested that structural alterations were not a major contributor to the increased resistance found in S rats compared to R. Sensitivity to norepinephrine was not different between the strains at any time during the exposure to high salt or prior to it. Thus, structural alterations seen in hypertensive S rats may be secondary to

the hypertension itself, rather than a primary cause for the increased vascular resistance seen in these rats.

The genetic difference between Dahl S and R rats has not been fully characterized. It is clear, however, that part of the predisposition to salt-induced hypertension in S rats lies in their kidneys. Evidence from several laboratories has pointed out an inherent deficiency in sodium excretion in S rats as compared to their R rat controls. The following three studies illustrate this point. Although there is also evidence that the decreased natriuretic capacity in S rats may be conferred by a circulating substance (Tobian 1979), the defect in sodium excretion is present in isolated, artificially perfused S rat kidneys as well.

Tobian et al. (1978) demonstrated decreased natriuretic capacity and renin release in kidneys of S rats as compared to R rat kidneys. Isolated kidneys from normotensive S or R rats on low salt were perfused with blood from normal rats and sodium and water excretion were measured at selected perfusion pressures. At 130 mmHg pressure, S rat kidneys excreted 52% less sodium than similarly perfused R rat kidneys. A similar difference was apparent at 160 mmHg, however at this perfusion pressure, S rat kidneys were able to excrete sodium at a rate equivalent to that of an R rat kidney perfused at 130 mmHg. Similar results were noted with respect to urine excretion. This evidence suggests that the kidneys of S rats have reset to maintain normal GFR but at a higher inflow pressure. At normal blood pressure, this deficiency in sodium and water excretion would predispose S rats to salt and water retention and a resultant expansion of their extracellular fluid volume. Therefore the S rat would be unable to excrete excess salt at normal

blood pressure and only achieve fluid volume maintenance when its blood pressure is elevated.

Similar results were obtained by Girardin et al. (1980) using isolated S or R rat kidneys perfused with ox red blood cells. Five groups of rats were examined. One R and one S group were maintained on a low sodium diet (0.02%) until kidney isolation. One R and one S group received a high sodium (8.02%) diet for seven weeks. The fifth group was S rats placed on high sodium for only three weeks. Urine excretion, sodium excretion and GFR were measured in isolated kidneys from each group in response to alterations in perfusion pressure. Following seven weeks of high sodium, kidneys from the hypertensive S rats showed decreased sodium excretion and GFR when compared to R kidneys at the same perfusion pressure. Raising the perfusion pressure in S kidneys could overcome this defect in sodium excretion, but GFR was still lower in S kidneys than in R even at 185 mmHg perfusion pressure. No difference was detected between S and R when both had remained on the low sodium diet. In addition, this difference was not detected after only three weeks of high sodium in S rats when their blood pressure was approaching hypertensive levels. This finding of no pre-existing abnormality was in contrast with the findings of Tobian et al. (1978) where only rats on low salt diets were examined, and a significant difference was detected. The authors acknowledged this discrepancy and differences in perfusion media were suggested as a possible cause.

Maude and Kao-Lo (1982) also studied the difference between S and R rat kidneys. Four groups of rats were studied: S or R rats on low salt (0.04% NaCl) and S or R on high salt (8.0% NaCl). All kidneys were completely isolated and perfused with an aerated bicarbonate and saline

solution. Renal vascular resistance, GFR and sodium excretion were monitored at four different perfusion pressures. In the 81-100mmHg range, R kidneys had higher GFR than S but resistance and sodium excretion were not different. At the highest pressures, significant differences were detected in all three measured parameters. Kidneys from S rats on low salt were lower in renal vascular resistance, GFR and sodium excretion than from R rats on low or high salt intake. Kidneys from S rats on high salt were also lower than those from S rats on low salt. The authors concluded that the hypertension seen in S rats on high salt could be due to the abnormal propensity in their kidneys for salt and water retention.

The presence of this renal defect during low salt intake and normal blood pressure is less conclusive. Differences in perfusion methodology and in the sodium content of the low salt diets make interpretation difficult. However, two of the three studies did demonstrate that the abnormality exists prior to the exposure to high salt intake. This defect may have been masked in the study by Girardin et al. (1980) by their perfusion technique and the use of a low salt diet that was lower in sodium than in the other studies.

Fink et al. (1980) studied the renal vascular resistance in S and R rats on low salt (0.4%) and the changes in resistance that occur following high salt intake (8.0%). Renal vascular resistance, responses to renal sympathetic nerve section and stimulation, and responses to intra-arterial norepinephrine and Angiotensin II were determined. R rats showed a significant reduction of renal vascular resistance when placed on high salt for four weeks as compared to their R controls on low salt. Renal vascular resistance was identical in S rats on either

dietary regimen. Responses to nerve section were similar in both strains and were unaffected by dietary salt. S rats showed higher increases in resistance to intra-arterial Angiotensin II than R rats, but this difference wasn't affected by salt intake. The renal resistance response to norepinephrine was suppressed in R rats by high salt but not in S rats. It was concluded that the S rats showed inappropriately high renal vascular tone in response to high salt intake. This alteration was apparently not the result of increased nerve activity or increased vascular reactivity to norepinephrine or Angiotensin II. It is therefore apparent that some intrinsic mechanism in S rats prevents them from the normal response of renal vasodilation in response to increased dietary salt as seen in R rats. Responses of the extrarenal vasculature to salt loading show this same pattern as well (Ganguli et al., 1979).

This intrinsic renal difference in salt handling and renal vascular resistance could be due to genetic differences in catecholamine metabolism between the two strains. Fernandez-Pardal and Saavedra (1982) demonstrated differences between S and R rats with respect to catecholamine levels in various regions of the kidney. Assay of frozen tissues obtained by a punch technique on kidney sections was performed on S or R rats on low or high salt intakes. S rats had higher levels of epinephrine than R rats in both the inner and outer medulla and the inner and outer cortex. Norepinephrine levels were different also, where S rats had higher levels than R, however, R rats showed increased norepinephrine and S rats decreased, in response to high salt intake. This difference in responses to salt was significant only in the outer medulla. Dopamine levels were different in the inner cortex and outer

medulla, where S rats had higher levels than R rats. A high salt diet resulted in higher dopamine levels in the inner medulla, but only in R rats. The authors concluded that renal levels of all three catecholamines could be regulated by both genetic and dietary factors. This regulation depends on the kidney region considered, and suggests that intrinsic differences in catecholamine metabolism or synthesis could be involved in the pathogenesis of hypertension in the Dahl strain.

The above studies indicate several intrinsic differences between S and R rats. Relative to R rats, S rats seem to show inappropriate responses, or the lack of the normal responses, to increased dietary salt intake, and these possibly result in increased arterial blood pressure through an unknown mechanism. This increased pressure, by a mechanism of pressure diuresis, probably allows the S rat to excrete the excess salt that is retained as a result of their defect in renal sodium and water excretion. There is additional evidence that the intrinsic defect in fluid volume and blood pressure regulation is not confined solely to the kidneys in S rats. Central nervous system mechanisms involved in control of blood pressure may play a role as well.

Saavedra et al. (1980) demonstrated differences between S and R rats, when on high salt intake, in terms of enzymatic activity of the adrenaline-forming enzyme phenylethanolamine N-methyltransferase or PNMT in certain brain nuclei. PNMT activity was higher in an area called A2 in S rats than in R when both rats were fed high salt from weaning to 10 weeks of age. Furthermore, PNMT activity was lower in S than R in the area postrema and nucleus commissuralis. While both strains showed increases in PNMT activity in the A2 area when on high salt as compared

to low salt, there was a greater increase in S rats. These S rats also showed a greater decrease in PNMT activity than R rats in the area postrema and nucleus commissuralis when comparing low and high salt diets. Although the role of PNMT activity in brain nuclei is not well understood, this evidence supports the hypothesis of a central defect in blood pressure regulation in S rats. The authors were not able to determine whether the difference in PNMT activity could be secondary to the increased blood pressure, or involved in its development.

Gordon et al (1981) examined the baroreceptor reflex control of heart rate in conscious S and R rats on low or high salt diets. Graded doses of phenylephrine (0.25 to 4.0 micrograms/kg) or nitroglycerin (5 to 40 micrograms/kg) were administered following control hemodynamic measurements and the pressor or depressor responses, and the associated peak reflex changes in heart rate were monitored after each drug dose. Reflex tachycardia following nitroglycerin was not different in S or R rats on either diet. Tachycardia in response to phenylephrine was, however, greater in S rats than R when on either dietary regimen. Furthermore, reflex baroreceptor sensitivity, as measured by the peak change in heart rate, was lower in S than in R, when both were on low salt, in response to phenylephrine. This difference in sensitivity to blood pressure changes was not present when the animals were on high salt, although the curve for S rats was shifted to the right, indicating a resetting to function at a higher arterial pressure range. This defect present in S rats was shown to be due to altered baroreceptor sensitivity rather than increased reactivity to alpha agonists because following ganglionic blockade with Chlorisondamine (20mg/kg) the difference in responsiveness to phenylephrine challenge was not present.

Chlorisondamine itself produced a large drop in blood pressure in R rats, and an even greater decrease in hypertensive S rats, so that mean blood pressure was not different, following treatment, between the two strains. This difference in response was present whether expressed as absolute or percent change. The authors concluded that neurogenic factors play an important role in the early stages of hypertension in Dahl S rats. Furthermore, the decreased baroreceptor sensitivity in S rats was of genetic origin as it existed in prehypertensive S rats on low salt. These baroreceptor abnormalities appear to play a role in the increased pressor responsiveness in S rats.

Bunag et al. (1983) reported differences in central sympathetic function in Dahl S and R rats. Basal sympathetic tone was evaluated by the frequency of splanchnic nerve potentials and the hypotensive response to alpha adrenergic blockade with phentolamine. R rats on low salt showed less sympathetic tone and a smaller hypotension following alpha blockade than S rats on the same diet. Pressor responses to electrical stimulation of the ventromedial hypothalamus lead to greater increases in mean blood pressure in S than R rats. Peripheral responsiveness to vasoactive agents was probably not involved, because responses to norepinephrine, tyramine and vasopressin were not different between the strains. The central nervous system differences were present in normotensive S rats on low salt and indicate that genetically transmitted hyperactivity of sympathetic mechanisms in the hypothalamus may be involved in initiating hypertension in Dahl S rats.

In addition to differences in central neural pathways and the kidneys, Dahl S and R rats also differ in adrenal steroid hormone synthesis. Rapp and Dahl (1971) demonstrated marked differences in

adrenal steroidogenesis between S and R rats on either low or high salt diets. They reported a twofold greater ability to make the 18-hydroxy form of deoxycorticosterone (18OH-DOC) in S rats as compared to R rats. A decrement of equal magnitude in the ability of 11-beta hydroxylation of deoxycorticosterone to form corticosterone (B) was also demonstrated in S rats. These differences were expressed as an 18OH-DOC/B ratio which was high in S rats and low in R rats. When a population of normal Sprague Dawley rats (the parent strain of the Dahl S and R rats) was tested, both high and low phenotypes were found. Because the S phenotype (high 18OH-DOC/B ratio) was found in normotensive Sprague Dawley rats, it was concluded that the phenotype was probably not involved with sensitivity to salt. Salt resistance, however, may be conferred by the low ratio phenotype.

Evidence for a circulating vasoactive agent in Dahl hypertension

Evidence for the presence of a humoral factor possibly involved in the hypertension of S rats on high salt was presented by Dahl et al. (1969). In a series of experiments involving parabiosis (joining of the skin and subcutaneous tissue of two animals so that slow exchange of extracellular fluid is allowed) they examined whether hypertension in S rats could be conferred to untreated, normotensive S or R rats. S and R rats were joined by parabiosis in combinations to form three groups: S + S, S + R and R + R. These groups were placed on low (0.4%) or high (8.0%) sodium chloride diets. R + R pairs on low or high salt both remained normotensive. S + S pairs on low salt both developed mild hypertension while those on high salt all died of severe hypertension

within three months. R + S rats on low salt both remained normotensive. Two major findings were reported on the R + S pairs on high salt: 1) the R rat of the pair became hypertensive and, 2) the hypertension in the S partner was lower than that of a single (unparabiosed) S rat on high salt. A humoral factor was postulated by the authors that conferred susceptibility to hypertension from the S rat to its R partner. In a second series of experiments, S or R rats were made hypertensive by unilateral renal artery constriction with contralateral nephrectomy and joined in four parabiosis combinations with sham-operated S or R partners. In the S* + S group (where * denotes the hypertensive rat) both rats developed hypertension. In the R* + R, R* + S and S* + R groups only the operated rat became hypertensive while the sham-operated S or R partner remained normotensive. In addition, the hypertension in the operated rat in parabiosis was milder than in single (unparabiosed) operated rats, suggesting a protective effect by the sham-operated S or R partner. A sodium excreting hormone, present in both strains, but with hypertensinogenic properties when produced in S rats, was suggested by the authors.

No single known modulator of sodium excretion can wholly explain the salt retention and resultant hypertension in Dahl S rats. Investigation of the following known humoral agents has ruled out a primary role for them in the pathogenesis of this form of hypertension.

The renin - angiotensin - aldosterone system probably does not play a primary role in the hypertension of Dahl S rats. Renin release has been found to be 50% lower in S rats than in R rats, when both were on low salt intakes, in response to decreasing renal perfusion pressure (Tobian, 1978). In addition, increased renin release would be

inconsistent with the high arterial pressure and extracellular fluid volume expansion seen in hypertensive S rats.

Vasopressin levels do not seem to play a role either. Matsuguchi et al. (1981) investigated the role of vasopressin in Dahl hypertension. Radioimmunoassay for argenine vasopressin was used to determine plasma levels, and pressor sensitivity was evaluated by intravenous injection of argenine vasopressin. The contribution of vasopressin to the hypertension was evaluated by injection of 50 micrograms of $d(CH_2)_5$VDAVP, a specific blocker of the pressor action of vasopressin. The plasma levels of vasopressin were elevated in both S and R rats when on high salt, but were higher in S rats. The levels were not different between S and R rats when on low salt. Pressor responses to intravenous argenine vasopressin were greater in S rats than in R, and there was no difference in responsiveness between S rats on low and high salt diets. Intravenous injection of $d(CH_2)_5$VDAVP failed to lower blood pressure in S rats on high salt. The dose was, however, sufficient to prevent pressor responses to exogenous argenine vasopressin. The authors concluded that vasopressin, although elevated in hypertensive S rats, did not play a major role in this type of hypertension.

Limas et al. (1981) measured vascular and renomedullary prostaglandin synthesis and degradation in Dahl S and R, and Sprague Dawley rats. Synthesis was evaluated by measuring incorporation of ^{14}C arachidonate, and degradation by breakdown of ^{3}H-PGE_2. Aortic PGI_2 synthesis was increased in response to high salt in S, but not R or Sprague Dawley rats. High salt intake increased renomedullary PGE_2 in all three strains, with S rats showing the smallest increase.

Degradation of PGE_2 in renal cortex by 15-hydroxyprostaglandin dehydrogenase was lowest in R rats and increased with salt loading in S and Sprague Dawley rats. The authors interpreted these findings as demonstration that the net renal output of prostaglandins was highest in R rats and lowest in S rats, and suggested that this difference may be relevant to the increased sensitivity to salt seen in S rats.

Faldareau and Martineau (1983) measured the 24 hour urinary excretion of 2,3,dinor-6-oxo-$PGF_{1\ alpha}$ and 2,3,dinor-13,14-dihydro-6,15-dioxo-$PGF_{1\ alpha}$, the two major metabolites of PGI_2, in Dahl S and R rats, by gas chromatography and mass spectrometry. The levels of these metabolites was used as an index of in vivo PGI_2 production. Control levels of PGI_2 were not found to be different between the strains prior to increased salt intake. Following administration of the high salt diet, PGI_2 synthesis increased significantly in R rats while decreasing in S rats. These findings are in contrast with those of Limas et al. (1981) where increased PGI_2 synthesis in response to high salt in S rats was reported. The role of prostaglandins in Dahl hypertension is still controversial. More work is necessary in this area in order to establish the roles of the various prostaglandins in the renal handling of a salt load.

Carretero et al. (1978) measured urinary kallikrein excretion in S and R rats by radioimmunoassay and enzymatic methods. In both techniques, kallikrein excretion was lower in S rats than R rats when both remained on a low (0.45%) sodium diets. Since kallikrein excretion is decreased in human essential hypertension and most forms of experimental hypertension, and is involved in water and electrolyte excretion (Carretero and Scicli, 1981), these findings were interpreted

as demonstration that a genetic defect in kallikrein excretion may predispose Dahl S rats to volume expansion and hypertension through alterations in renal sodium and water excretion. The responses to increased dietary salt were not examined in this study.

Sustarsic et al. (1980) extended these studies to include S and R rats on high salt (8.05) diets, and semipurified the urinary kallikrein prior to assay to remove interfering substances. Contrary to the findings of Carretero et al. (1978) these investigators did not find a difference between strains when on low salt diets. When on high salt diets, both strains showed decreased kallikrein excretion, but the decrease was greater in S than R rats. Total urinary protein was higher in S rats also, and the authors suggested that the lower urinary kallikrein excretion in S rats may be secondary to hypertension and renal damage rather than a causative factor in the hypertension.

The above studies suggest the lack of a primary role for the renin-angiotensin, vasopressin, prostaglandin and kallikrein-kinin systems in the genesis or maintenance of hypertension in the Dahl S strain on high salt. However, several lines of evidence suggest that catecholamines may be intimately involved in the hypertensive process in this strain.

Takeshita and Mark (1978) investigated the contribution of the sympathetic nervous system to the vascular tone of the isolated hindquarters of S and R rats on low or high salt diets. Five major conditions were studied: 1) vascular resistance with innervation intact, 2) responses to direct sympathetic nerve stimulation, 3)vascular resistance following denervation, 4) pressor responses to norepinephrine, and 5) resistance during maximal vasodilation by

papaverine. On low salt diets, S and R rats showed no differences in vascular resistance, the response to norepinephrine or to sympathetic nerve stimulation. S rats showed higher resistance when hypertensive and on high as compared to low salt diets. Neurogenic tone, as measured by the difference in resistance before and after denervation, was higher in S relative to R rats as well. Hindquarter vasoconstrictor responses to sympathetic nerve stimulation were higher in S rats on high salt than those on low, but the responses to norepinephrine were not different. R rats on high salt were not different from those on low salt in any of the measured parameters. The interpretation by the authors was that neurogenic mechanisms account for 50% of the increased hindquarter resistance in S rats on high salt because the fall in resistance following denervation in hypertensive S rats on high salt accounted for 50% of the difference between them and control S rats on low salt. In addition, there was augmentation of the response to sympathetic nerve stimulation in S rats on high salt that was apparently not due to enhanced sensitivity to norepinephrine.

The dependence, of the elevated vascular resistance in hypertensive S rats, on the presence of an intact sympathetic nervous system was further demonstrated by Takeshita et al. (1979). Dahl S and R rats were treated with 6-hydroxydopamine (75-100 mg/kg intraperitoneally, twice weekly) to achieve full chemical sympathectomy. The efficacy of the sympathectomy was tested by electrical stimulation of the lumbar sympathetic chain and by i.v. injection of tyramine. Following one week of treatment, and the same period of vehicle treatment in control S rats, both groups were placed on 8.0% sodium chloride diets for seven weeks. After this time, hindquarters vascular

resistance, sympathetic tone and responses to sympathetic nerve stimulation were evaluated. S rats treated with 6-hydroxydopamine failed to become hypertensive, while hypertension developed normally in vehicle-treated controls. Hindquarters vascular resistance was significantly lower than in the controls, and lumbar sympathetic denervation produced no effect on resistance. Responses to sympathetic nerve stimulation and tyramine were also 70-80% less than in the vehicle-treated control S rats. Responses to norepinephrine administration were not different between control and the 6-hydroxydopamine treated rats. These results demonstrate that an intact sympathetic nervous system is necessary for the development of hypertension in Dahl S rats.

Friedman et al. (1979) studied the effects of neonatal sympathectomy with guanethidine on the response of S rats to high salt. Guanethidine (50 mg/kg intraperitoneally) was administered five times per week for three weeks starting eight days after birth. Control S rats received saline injections. Following the last of the 15 injections, the rats were placed on low (0.4%) or high (8.0%) sodium chloride diets. Sympathectomized rats did not develop hypertension. The efficacy of sympathectomy was tested by assaying tissue catecholamine levels. In addition, direct blood pressure measurement and the responses to Angiotensin II, tyramine, norepinephrine and pentolinium (a ganglionic blocking agent) were evaluated. The response to tyramine was lower in sympathectomized rats regardless of dietary sodium chloride intake. The blunted response to tyramine was not due to a generalized loss of vascular reactivity because angiotensin II produced greater responses in sympathectomized rats compared to control

rats. Norepinephrine produced responses similar to those for angiotensin II. Pentolinium caused a greater vasodepression in control rats as compared to sympathectomized rats similarly fed. The authors' conclusions were that guanethidine had successfully caused a peripheral sympathectomy and that the absence of sympathetic nerves prevents the hypertension from developing.

Altered sympathetic tone to the vasculature of hypertensive S rats on high salt seems to be involved in the hypertension of these rats. The increased tone is apparently not due to enhanced sensitivity to norepinephrine (Mueller, 1983) and seems to be mediated through a central sympathetic mechanism (Bunag, 1983). The increased vascular tone due to enhanced sympathetic outflow is responsible for 50% of the increase in resistance seen in these rats (Takeshita and Mark, 1978). The cause of the remaining component of the increased resistance remains unknown and is probably not dependent on other known vasoactive agents. The role, if any, of ANF and the ouabain-like factor (OLHF) has not been investigated with respect to the Dahl strain of rats. Both have been shown to be involved in fluid volume and blood pressure regulation as well as involved in the development and/or maintenance of hypertension in some experimental models. The role of OLHF has not been previously evaluated in the established phase of hypertension in Dahl S rats on high salt. Simultaneous with this research project, several laboratories are investigating the role of ANF in this genetic model of hypertension.

Hirata et al. (1984) measured atrial levels of ANF in Dahl S and R rats by injecting boiled atrial supernates from S or R rats on three different dietary regimens into normal Sprague Dawley bioassay rats.

The maximal increase in sodium excretion was used as an index of the levels of ANF in the atria. Rats were tested following low salt (0.11%), five days of high salt (4.0%) and following 12 weeks of high salt. Atria of S rats on high salt contained more ANF than those from R rats on the same diet. This difference between strains was apparent even when the rats remained on low salt diets. Increases in atrial ANF levels were reported after five days of high salt in R rats. These increases in ANF were not present, however, after 12 weeks of high salt. S rat atria contained similar amounts of ANF regardless of salt intake. In a second set of experiments, pooled atrial extracts from normal Sprague Dawley rats were infused into S or R rats which had been maintained on low salt. S rats showed a much smaller increase in natriuresis than R rats receiving the same amount of supernate. This finding indicates that the S rat may be less sensitive to ANF than the R rat. Lastly, atrial and ventricular supernates were infused into nephrectomized and sham-nephrectomized rats to measure effects on blood pressure. Similar decreases of approximately 20 mmHg were observed in both rats. These findings demonstrate an effect on blood pressure that is independent of any loss of water and electrolytes through natriuresis, and again suggest a direct vascular effect as well.

PURPOSE OF THE INVESTIGATION

Evidence from ours and other laboratories suggest that the two natriuretic factors namely: 1) the ouabain-like humoral factor (OLHF) and 2) Atrial Natriuretic Factor (ANF) may play a role in the development and maintenance of low renin, volume expanded hypertension. Their levels have been shown to be altered in several experimental hypertension models and in response to acute or chronic changes in body fluid volumes. We wondered whether these substances were similarly involved in the Dahl model of salt-dependent hypertension. Higher levels of OLHF, if present, could partly explain the hypertension seen in Dahl S rats on high salt diets through the mechanism of depression of vascular $Na^+ - K^+$ pump activity or alterations in Na^+/Ca^{++} exchange as mentioned previously. Conversely, lower levels of ANF, or depressed sensitivity to its effects, could also explain, in part, the salt retention, elevated vascular resistance to blood flow, and hypertension in these rats. Altered sensitivity to these natriuretic agents could also be involved.

The objectives of this investigation were to: 1) examine plasma levels of OLHF, among the four groups of Dahl strain rats, by comparing the effects of boiled plasma supernates from these rats on the uptake of ^{86}Rb (an index of $Na^+ - K^+$ pump activity) in normal rat tail arteries; 2) investigate the role of variations in dietary salt intake on the plasma levels of OLHF, again as determined by the effects of plasma supernates on ^{86}Rb uptake in normal tail arteries; 3) to determine relative atrial content of ANF, among the four groups of Dahl rats, by bioassay of atrial extracts for natriuretic and diuretic activity in

normal assay rats; 4) to determine atrial levels of ANF by radioimmunoassay for AP III-like immunoreactivity; 5) to compare the relative potency of atrial extracts from Dahl rats for their effects on norepinephrine-induced contractions in aortic rings from normal rats; 6) to investigate the renal responsiveness, to exogenously applied ANF, in normotensive Dahl S ans R rats consuming low salt diets, by i.v. injection of atrial extracts from normal rats into S or R assay rats and comparison of the natriuretic and diuretic effects; 7) to investigate the vascular responsiveness to ANF, in the Dahl strain, by comparing aortic rings from normotensive S and R rats consuming low salt diets for the effects, of exogenously applied ANF, on their contractile response to norepinephrine.

METHODS

Preparation of hypertensive Dahl S rats and their controls

All Dahl strain S and R rats used in these studies were males obtained as weanlings (approximately four weeks old) weighing 100 to 150 grams from Brookhaven National Laboratories. Upon arrival, all rats were placed on a grain-based, low (0.4% NaCl) salt diet (Purina #5001-3) for two weeks, during which time normal blood pressure was documented in all rats. Diets containing this level of salt content were used because the normally available rat chow contains 1.0% NaCl and Dahl S rats would develop mild hypertension at this level of salt intake. Individuals of either strain having systolic blood pressure higher than 140 mmHg at this time were rejected from use in any studies. Blood pressures were determined in the conscious state using a tail plethysmograph (Natume model KN-209) and were monitored weekly throughout the studies. Following two weeks of documented normotension and maintenance on the low salt (0.4% NaCl) diet and tap water ad libitum, the rats were divided into four groups: two S groups and two R groups. One S group and one R group remained on the low salt diet (S_{LS} and R_{LS} respectively) throughout the study. The remaining S group and R group rats (S_{HS} and R_{HS} respectively) were placed on a high salt diet (8.0% sodium chloride, Purina #5001-1). As expected, S_{Hs} rats developed hypertension. After four to six weeks of sustained hypertension (systolic BP>140 mmHg), and a similar time period for control rats, the rats were sacrificed for use in the following experimental procedures.

Protocol using synthetic sodium deficient diets

Our initial determinations of plasma OLHF levels did not show any difference in OLHF content in plasma supernates from S_{Hs} rats relative to normotensive S rats on low salt diets (S_{LS}). Because salt excretion is impaired in S rats (Tobian et al. 1978), it is possible that the ouabain-like factor was present due to volume expansion not only in the S rats on high salt, but also in the S rats on low salt. This expansion could stimulate release of the ouabain-like factor in S rats consuming low salt in quantities not sufficient to cause hypertension. To rule out this possibility, we tried a diet lower in salt content in control groups to prevent salt retention and volume expansion in the S rats on low salt. A synthetic sodium-deficient diet containing only 0.04% NaCl (Bio-Mix #101, Bioservices Inc.) was substituted for the grain-based, low (0.4%) salt chow in all groups of Dahl rats from weaning, and for the initial two week control period. One group of S rats and one group of R rats remained on this diet for the rest of the study. The remaining groups (one S and one R) consumed the same diet plus 8.0% added sodium chloride (w/w). They remained on this diet until the S rats were hypertensive for four to six weeks as per the normal protocol. Plasma supernates were then prepared from these rats and assayed for the presence of the ouabain-like factor.

ASSAY FOR OLHF IN PLASMA OF DAHL S AND R RATS

Preparation of boiled plasma supernates for detection of OLHF

Following four to six weeks of hypertension (systolic BP>140 mmHg) in S rats on high salt, and the same time period for the three control groups, the rats were anesthetized with sodium pentobarbital (75 mg/kg, intraperitoneal). The abdomen was opened by midline incision and the abdominal aorta exposed and cleared of adventitia. Six milliliters of blood were collected, by direct aortic puncture, into a pre-chilled syringe surrounded by an ice jacket. The blood was immediately transferred into a chilled tube containing 0.2 ml sodium heparin (Porcine, 1000 Units/ml) and centrifuged at 1100 x G for ten minutes in a refrigerated centrifuge (Sorvall RC-5, SM-24 rotor). The plasma was collected, placed in 16 x 125 mm screw cap glass tubes and incubated at room temperature for 30 minutes to enhance generation of the $Na^+ - K^+$ pump inhibiting factor if any was present. Earlier studies by Gruber et al. (1978) have demonstrated that the activity of OLHF is maximized in this manner, possibly by proteolytic degradation of larger precursors into the active form. The plasma was boiled in the same tube for five minutes in a covered water bath at 100 degrees C to destroy any proteolytic activity that could inactivate the OLHF. The resultant viscous material was macerated with a glass rod and transferred to a polycarbonate high speed centrifuge tube and centrifuged at 36,000 x G for 90 minutes in the same refrigerated centrifuge used above. The supernate was collected (1.0 to 1.5 ml) and frozen at minus 20 degrees C until used to assay for the presence of the ouabain-like factor. Plasma supernates were assayed within one to two weeks of preparation.

Additional remaining supernate was used for chemical analysis of its composition.

Assay of boiled plasma supernates

Boiled plasma supernates were assayed for ouabain-like activity using the ^{86}Rubidium uptake method (Pamnani et al., 1978; 1981). Two normotensive male Sprague Dawley rats weighing 350 to 400 grams were anesthetized with sodium pentobarbital (75mg/kg, intraperitoneal). Their tail arteries were gently excised, cleaned of adventitia, opened longitudinally and cut into eight pieces, each eight to ten millimeters in length. The pieces were incubated 10 to 15 minutes in aerated (95% O_2, 5% CO_2) Krebs - Henseleit solution to allow recovery from the surgical manipulation. The composition of the Krebs - Henseleit solution was as follows: 118.0 mM NaCl, 27.2 mM $NaHCO_3$, 4.8 mM KCl, 1.0 mM KH_2PO_4, 1.2 mM $MgSO_4$, 11.1 mM glucose and 2.5 mM $CaCl_2$. Following this incubation, the pieces were divided into four groups of one half an artery each and assigned so each group contained representative portions of the length of a whole tail artery and contained portions of the artery of each rat. The four groups of artery pieces were then incubated in randomly ordered plasma supernates from the four groups of Dahl rats for two hours at room temperature. Supernate volume was one milliliter. At the end of this incubation, radioactive ^{86}Rubidium (as ^{86}RbCl, 0.01 to 0.03 mM final concentration; specific activity equal to 1.0 to 10.0 mCi/mg) was added to each tube and the incubation was continued for 18 minutes at 37 degrees C in a shaker water bath with intermittent aeration (95% O_2 :5% CO_2). Following incubation with the

isotope, the tissues were removed from the tubes and quickly rinsed sequentially in three 50 ml volumes for ten seconds each in K^+-free Krebs-Henseleit solution containing non-radioactive RbCl (117.0 mM NaCl, 27.2 mM $NaHCO_3$, 1.0 mM NaH_2PO_4, 1.2 mM $MgSO_4$, 11.1 mM glucose, 2.5 mM $CaCl_2$ and 2.0 mM RbCl). This rinsing removes extracellular ^{86}Rb and prevents further uptake of the isotope. The tissues were then blotted dry gently on lint-free towels and counted on a gamma counter (Searle 1185) with the appropriate standards containing known amounts of ^{86}Rb and background blanks consisting of empty counting tubes. Following counting, the artery pieces were dried for 24 hours in a drying oven at 100 degrees C and weighed. ^{86}Rb uptake was calculated as counts per minute divided by specific activity, per mg of tissue, and expressed as pMoles ^{86}Rb/mg tissue (dry wt.).

Chemical composition of boiled plasma supernates from Dahl rats

The boiled plasma supernates used for assay of $Na^+ - K^+$ pump activity were also assayed for chemical composition to rule out any possible difference in composition that could affect pump activity independent of OLHF or have an effect on hemodynamics and blood volume. Na^+ and K^+ concentrations were measured by flame photometry (Beckman Klina Flame). Ca^{++} and Mg^{++} concentrations were determined by atomic absorption spectrophotometry (Perkin Elmer 603) and chloride concentration by titration (Radiometer, Copenhagen, CMT 10 Chloride Titration). Osmolality was measured by freezing point depression (Advanced Instruments, Inc., Model 3D II), protein concentration by the biuret method, blood urea nitrogen by colorimetric methods (Davidson and

Wells, 1962), and creatinine levels by the method of Bonsnes and Tausky (1945) using a Beckman Model 26 Spectrophotometer.

ASSAY FOR ATRIAL CONTENT OF ANF IN DAHL RATS

In order to examine whether different levels of ANF could contribute to the hypertension seen in Dahl S rats on high salt, we sought to measure the atrial content of ANF in these rats relative to S_{LS}, R_{HS} and R_{LS} rats. Three separate methods were used to quantify ANF-like activity in extracts of atrial tissue from these rats. The atrial extracts were prepared on a per weight basis as detailed below to normalize for differences in atrial tissue sample weights.

Preparation of atrial extracts for natriuresis - diuresis bioassay

Atrial tissue extracts were prepared using the method of Thibault et al. (1983). Following four to six weeks of hypertension, Dahl S rats and their three controls (R_{HS}, S_{LS} and R_{LS}) weighing 350 to 450 grams, were anesthetized with sodium pentobarbital (75mg/kg, intraperitoneal) and their hearts rapidly excised and placed in 5mM phosphate-buffered 0.9% saline (pH 7.40) on ice. All blood was thoroughly rinsed away and the left and right atria with attached atrial apppendages were dissected free. Atria from each rat were processed individually. Following dissection, each atrium (consisting of both right and left atrial appendages and weighing from 50 to 100 mg) was blotted to remove excess saline and weighed. The tissue was then homogenized for 30 seconds (Brinkman Polytron, speed setting 8) in 2.5

milliliters of 1.0 N acetic acid on ice. The homogenate was centrifuged at 15,000 x G for 20 minutes in a refrigerated centrifuge (Sorvall RC-5, SM-24 rotor), and the supernate collected and kept on ice. The pellet was then resuspended in an additional 2.5 milliliters of acetic acid and recentrifuged as above. The resultant supernate was pooled with the initial one and together they were shell frozen in a dry ice and acetone mixture and lyophilized at -60 degrees C at 50 microTorr vacuum. Following the lyophilization, the powdered material was resuspended in 0.1 N acetic acid to a final concentration of 1:6 w/v (original atrial tissue wet weight: acetic acid) and transferred to 17 x 100 mm polycarbonate tubes. The tubes were left to stand on ice for approximately two hours with repeated mixing to fully resuspend the lyophilized material and centrifuged again at 15,000 x G in the same centrifuge. The final supernate was collected and frozen at -70 degrees C until used for assay of natriuretic and diuretic activity. Any additional precipitate that formed upon thawing was removed by centrifugation again at 15,000 x G for 20 minutes. Atria from normal Sprague Dawley rats were pooled and processed in the same manner for use in natriuresis-diuresis bioassays using normotensive Dahl S or R rats on low salt diets as assay animals.

Preparation of atrial extracts for bioassay and radioimmunoassay

During the course of these studies, more efficient methods for extraction of ANF activity were developed in our laboratory. It has been shown by radioimmunoassay that extraction with 1.0 N acetic acid provides a higher yield of ANF than the method using 0.1 N acetic acid and lyophilization described above (Hom et al., 1985). Although this method could not be utilized for i.v. injection into intact animals because the injection of 1.0 N acetic acid was not well tolerated in bioassay rats, the 1.0 N preparation was well suited for use in the contractility bioassays and in radioimmunoassay procedures.

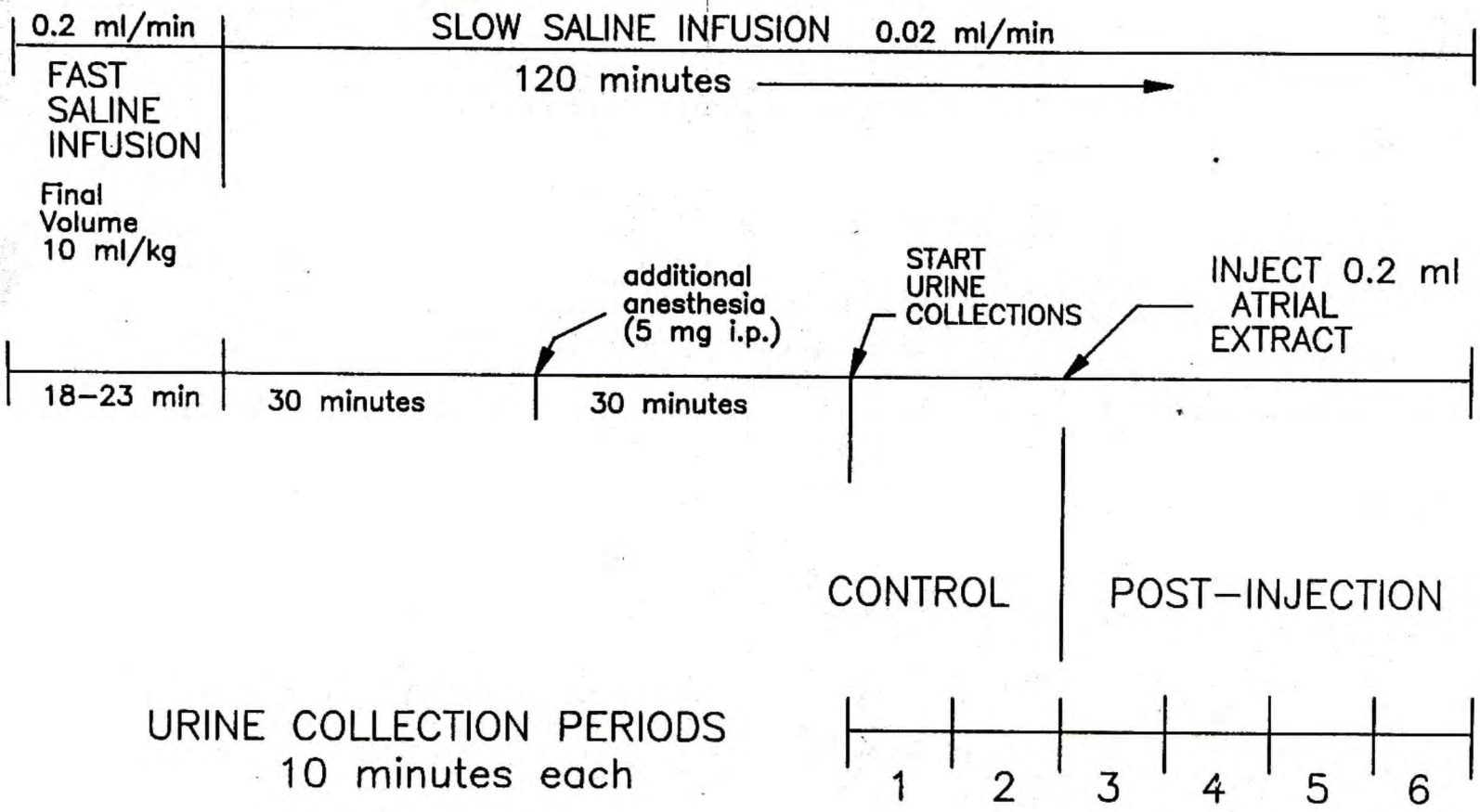
Atria were collected from pentobarbital anesthetized S and R, or normal Sprague Dawley rats, blotted to remove traces of blood, and immediately frozen on dry ice. The frozen tissue was weighed, mixed 1:10 in 1.0 N acetic acid (0.1 grams atria per ml acid), and homogenized for 30 seconds (Brinkman Polytron, speed setting 8). The resultant suspension was centrifuged at 36,000 x G for 30 minutes (Sorvall RC-5, SM 24 rotor), the supernates were collected and frozen at -70 degrees C for later use. Atrial homogenates from Dahl S or R rats were diluted 1:20 instead of 1:10 to facilitate the homogenization because of the small volumes used in the preparation of individual rat atria (Sprague Dawley atrial extracts were prepared from pooled atria from several rats). Similarly prepared extracts from the apex of the ventricles of Sprague Dawley rats were used as controls for experiments on aortic contractility as these ventricular extracts have been shown not to contain ANF (de Bold et al., 1981).

Assay of Dahl atrial extracts by natriuresis - diuresis bioassay

The diuretic and natriuretic responses induced by atrial extracts from the Dahl rats were evaluated as follows. A normotensive Sprague Dawley rat weighing 350 to 450 grams was anesthetized with sodium pentobarbital (75mg/kg, intraperitoneal) and the neck and inguinal regions shaved. A tracheal cannula (Intramedic PE-240) was inserted to facilitate respiration, and an intravenous catheter (Intramedic PE-50) prefilled with 0.9% saline containing heparin (10 Units/ml) was introduced into the external jugular vein and tied in place with suture. Through a suprapubic incision the bladder was gently exposed. A flared end catheter (Intramedic PE-90) was inserted through a small incision in the body of the bladder and tied in place with suture. An additional catheter (Intramedic PE-50) was placed in the peritoneal cavity to facilitate administration of additional anesthesia, and together both catheters were routed out through the suprapubic incision which was then sutured closed. Following a 15 minute interval to allow for recovery from the surgical manipulations, the rat was infused intravenously with 0.9% saline, 10ml/kg body weight, at a rate of 0.2 ml/minute (Harvard Apparatus infusion pump, model 940) for 17 to 23 minutes. After this fast infusion the rate was reduced to 0.02 ml/minute and maintained at this rate for the next 120 minutes. A schematic representation of the procedure is presented in Figure 2. One half hour into this infusion the rat was given an additional dose of 5 mg sodium pentobarbital to maintain the anesthetic state. Thirty minutes later, control urine volume (UV) was determined by collecting two samples of ten minutes each. Atrial extract, 0.20 milliliters as a bolus intravenous injection, was given following the control urine

FIGURE 2. Schematic of the time frame for the Natriuresis - Diuresis bioassay for atrial ANF content. Infusion periods and urine collection intervals are shown. Assay animals were normal Sprague Dawley rats.

NATRIURESIS – DIURESIS BIOASSAY



volume collections and urine collections continued at ten minute intervals for the next 40 minutes. Urine volume was determined gravimetrically and expressed as microliters per minute. Urinary sodium concentration was determined by flame photometry (Beckman Klina Flame) and expressed as microequivalents Na^+ excretion ($U_{Na}V$) per minute.

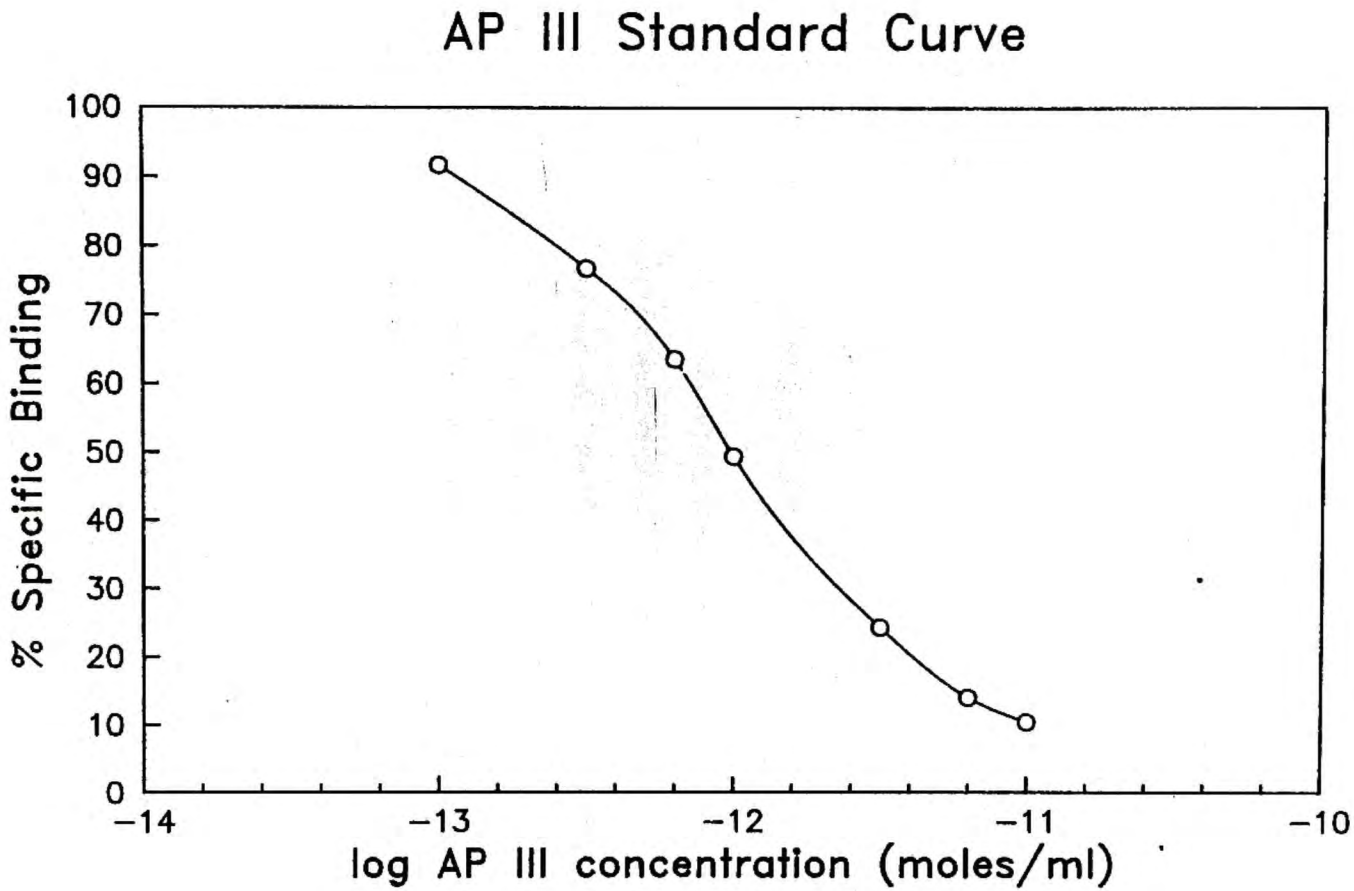
Radioimmunoassay for levels of ANF in Dahl rat atrial extracts

The radioimmunoassay procedure used in these studies was developed in the laboratory of Dr. Gregory Mueller. Antibody to Atriopeptin III was developed in rabbits against the C-terminal heptapeptide of AP III obtained from Peninsula Laboratories Inc.. The amino acid sequence was LYS-CYS-ASN-SER-PHE-ARG-TYR. The peptide was conjugated to bovine thyroglobin, emulsified with complete and incomplete Freund's adjuvant and injected intradermally into white New Zealand rabbits. Boosting injections were given at four to six week intervals and serum was collected periodically to determine the antibody titer. The antisera from the rabbit, labeled X-80, had the highest titer and sensitivity to AP III and did not cross-react with AP I or AP II, indicating an absolute requirement for the C-terminal Tyrosine residue. At a dilution of 1:50,000, it bound 30-40% of radiolabeled AP III and was sensitive to less than 10 femtamoles of AP III standard. The radioactive trace used in the assay procedure was obtained by iodination, with ^{125}I, of the same C-terminal heptapeptide on the Tyrosine residue. The trace was then purified by reverse phase chromatography on Sep-Pak cartridges (Waters Associates, Inc.). The antibody binds the trace in the absence of AP III yielding a Total

Binding value. The presence of AP III in a sample displaces some of this binding by its own binding to antibody and prevents the trace from being totally bound. Unbound trace is removed in the final step by charcoal which is centrifuged out leaving only the bound trace in the supernate which is counted.

The assay procedure was as follows. Atrial extract samples (prepared in 1.0 N acetic acid) were diluted with phosphate buffer (0.05 M PO_4^{-3}, 0.05% Bovine Serum Albumin, 0.02% NaN_3 and 5 mg% Bacitracin) to achieve 1:800, 1:1600 and 1:2400 dilutions. These dilutions were chosen following independent assay of pooled atrial extracts in order to determine the dilutions which were on the linear portion of the standard curve. Assay volume was 0.1 ml for atrial extract samples and standards. The standards used contained 10, 30, 60, 100, 300, 600 and 1000 fmoles of AP III standard (Peninsula Laboratories). The standard curve is shown in Figure 3. Concentrations of atrial extract yielding specific binding between 25% and 75% were used in determining AP III levels. Tubes for Total Counts contain trace only, while those for Non-Specific Binding contain no antibody and measure binding of trace not caused by antibody. Two hundred microliters of phosphate buffer was added to all tubes except the Total Counts tube (0.1 ml trace only), while the Non-Specific Binding tubes received an additional 0.1 ml buffer to account for the absence of the 0.1 ml of antibody. The Total Binding tube received an additional 0.1 ml buffer to account for the absence of AP III standard. One hundred microliters of trace was added to all tubes, except the Total counts tube, and each tube, except the Non-Specific Binding tube received 0.1 ml antibody. All tubes were run in duplicate. Following 24 hours of incubation in the refrigerator, all

FIGURE 3. Displacement curve for AP III standards. Increasing concentration of standard displaced binding of radioactive trace to the antibody, while unbound trace is removed, yielding lower specific binding of the trace.


AP III Standard Curve
% Specific Binding
100
90
80
70
60
50
40
30
20
10
0
−14
−13
−12
−11
−10
log AP III concentration (moles/ml)

tubes (except the Total Counts tube) received 1.0 ml of charcoal suspension (1:10 Bovine Serum Albumin: Charcoal, 0.55 grams per 100 ml buffer). The buffer composition was 12.0 mM NaH_2PO_4 plus 38.0 mM Na_2HPO_4 in distilled water. The resulting suspension was centrifuged at 2200 rpm for 45 minutes in a refrigerated centrifuge (Sorvall RC-3B, hanging bucket). The supernate was decanted into 12 x 75 mm glass tubes, covered with Parafilm and counted on a gamma counter (Searle 1185). Immunoreactive AP III in the samples was determined by comparison to the standard curve, after the counts due to non-specific binding were subtracted.

Bioassay for ANF content by effects on aortic contractility

Aortae from normotensive male Sprague Dawley rats were used in all experiments where vasodilator activity of atrial extracts prepared from Dahl S and R rats was determined. Rats weighing between 350 and 450 grams were decapitated and the thoracic aortic segment from the arch to the diaphragm was gently dissected and cleaned of blood and adventitia. Segments 2.5 mm in length were cut by scalpel yielding rings that were immediately placed in an organ bath containing 25 ml Krebs - Ringer solution aerated with 95% O_2 and 5% CO_2. The composition of the Krebs - Ringer solution was 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$ and 5.6 mM glucose (pH 7.40). The bath temperature was kept at a constant 37 degrees C by circulating through a thermostatically controlled water bath using a peristaltic pump. The rings were allowed to equilibrate for 15 minutes under zero tension, and then 1.0 grams of tension was applied. The

rings were left free to equilibrate at this tension for 45 minutes with a change of the bath solution at 15 minute intervals. Following this equilibration, the rings were exposed to repeated 10^{-8} M doses of norepinephrine (Levophed bitartrate, Breon Laboratories) until consistent tension responses were obtained while measuring developed tension using an isometric force transducer (Grass Instruments, FT03C) attached to the aortic ring by a stainless steel wire hook. The rings were thoroughly rinsed and allowed to relax completely between these norepinephrine doses. Developed tension was recorded on a paper chart recorder (Gould Brush 2200), calibrated to 20 mm = 1.0 gram. Following this equilibration and stabilization period, cumulative dose - responses to norepinephrine were performed by sequential addition of norepinephrine to achieve the following 12 final concentrations: 10^{-11} M, 10^{-10} M, 3×10^{-10} M, 10^{-9} M, 2×10^{-9} M, 4×10^{-9} M, 7×10^{-9} M, 10^{-8} M, 3×10^{-8} M, 10^{-7} M, 10^{-6} M and 10^{-5} M. After the maximum tension was developed (usually by 10^{-6} M norepinephrine concentration), the rings were rinsed several times and allowed to return to baseline tension. Following the return to baseline, 20 microliters of atrial extract from Dahl S_{LS}, S_{HS}, R_{LS} and R_{HS} rats was applied to the bath and the rings were allowed to equilibrate with the added extract for ten minutes. Then the dose - response curve was repeated using the same concentrations listed above.

Calculations of norepinephrine sensitivity

The concentration of norepinephrine that caused a contraction equal to 50% of the maximum tension developed by the ring is called the EC_{50} value. Rather than interpolating this value from a graph of the dose - response relationship (a procedure that only considers the two points bracketing this value), a method was developed that utilizes all the data points from the curve. Hill plots of the dose - response curves provided a straight line function, and linear regression analysis of the data points, following transformation using the Hill equation, gave an estimate of the EC_{50} value that was less dependent on statistical variations in single experiments.

The common form of the Hill equation is: $y = \log (Vmax/V - 1)$. Tension values at the various norepinephrine concentrations, in millimeters deflection on the chart recorder, were transformed by the following variation of the equation:

$$y = \log ((\text{max. tension}/\text{tension for a given dose}) - 1)$$

and plotted against the log of that given dose. Linear regression using the least squares technique provided the best fit equation for this straight line, and the log of the EC_{50} value was the value satisfying $y = 0$. An EC_{50} value for the norepinephrine response was calculated for each ring prior to, and following the addition of the atrial extract, and all values were expressed in nanomoles per liter.

This method was also used to evaluate the vasorelaxant effects of atrial extracts from normal Sprague Dawley rats. The effects of atrial extracts on the EC_{50} for norepinephrine were determined using several different concentrations of atrial extract. ANF dose-response curves were obtained by evaluating these effects of Sprague Dawley

atrial extracts, at concentrations of 8, 20, 40, 80 and 160 micrograms of atrial tissue per ml bath volume, on the EC_{50} of normal Sprague Dawley rat aortic rings. In addition, synthetic atriopeptins AP I, AP II and AP III (Peninsula Laboratories) were tested for vasorelaxant activity in the same manner. Concentrations ranging from 5 to 80 nanomoles per liter were used for each of these three synthetic atrial peptides.

EVALUATION OF THE RESPONSIVENESS OF DAHL RATS TO ANF

The determination of atrial levels of ANF in Dahl rats would be of little value unless their responsiveness to ANF was evaluated as well. Because our preliminary results showed elevated levels of ANF in Dahl S rats relative to R rats, an attempt was made to determine whether the difference in ANF level could be a secondary compensatory response due to altered sensitivity to ANF in these rats. Elevated levels may not be of consequence if the sensitivity is low and, likewise, lower levels of ANF may not be of physiological relevance if the sensitivity is high. Two methods were used to compare the sensitivity of Dahl rats to the natriuretic and vasorelaxant effects of atrial extracts obtained from normal rats.

Renal responsiveness in Dahl rats was evaluated by using the previously described natriuresis-diuresis bioassay. The diuretic and natriuretic response to i.v. injection of equal amounts of atrial extract was used as an index of the sensitivity of these Dahl rats to the renal effects of ANF. Normotensive male Dahl S and R rats fed only low salt diets (0.4% NaCl: systolic BP for S rats 132 ± 1 mmHg, for R

rats 120 ± 1 mmHg) were used as bioassay animals. Only normotensive rats were used so that any difference in inherent sensitivity could be detected without the results being complicated by the presence of hypertension in the S rats. The rats were prepared for the assay in the same manner as described previously, and each received an intravenous bolus injection of 0.2 ml of atrial extract prepared from normal Sprague Dawley rats. The atrial extracts were prepared in the same manner as those used in the other natriuresis bioassays (in 0.1 N acetic acid). Urine collections were made by the method previously described and urine formation and sodium excretion were evaluated for each of the Dahl S or R assay rats.

Vascular sensitivity to ANF was determined using the previously described bioassay for aortic contractility. Our previous experiments had demonstrated a dose-dependent increase in the EC_{50} for norepinephrine-induced contractions of aortic rings from normal rats when exposed to atrial extracts. Aortic rings from normotensive Dahl S or R rats on low salt were prepared in the same manner and control EC_{50} was determined. Then the effect of various concentrations of atrial extracts from normal Sprague Dawley rats was evaluated. The EC_{50} for norepinephrine was determined in these aortic rings from S and R rats using four different concentrations of atrial extract (20, 40, 80 and 160 micrograms atrial tissue per ml bath volume).

Statistical methods

Comparisons among the four groups of S and R rats were made using analysis of variance using the Duncan's Multiple Range Test. P

values less than or equal to 0.05 were regarded as significant in all comparisons that were made.

RESULTS

I. EFFECTS OF DIETARY REGIMEN ON BODY WEIGHT AND BLOOD PRESSURE

A. Rats consuming grain-based diets.

All the animals studied remained healthy throughout the study as indicated by their general appearance, movements and smooth fur. The mean ± SEM of body weights for all four groups of Dahl S and R rats on low (0.4% NaCl) or high (8.0% NaCl) salt diets are presented in Table 1. Body weights were not significantly different before the beginning of the dietary regimen in Dahl S and R rats. The growth rate was similar during six weeks of the dietary regimen and there were no differences in body weight between the strains when consuming either salt intake, or when comparing rats on high relative to low salt intake within either strain.

Figure 4 shows systolic blood pressures recorded in the four groups. The actual numeric values are presented in Table 2 (see APPENDIX). At seven weeks of age, before the beginning of the dietary regimen, blood pressures of both S rat groups were significantly higher than that of either group of R rats. By the end of the first week of increased dietary salt intake, systolic blood pressure of S rats on high salt was significantly elevated from 129 to 145 mmHg and thereafter progressively increased to 170 mmHg by the sixth week of high salt intake. In contrast, R rats on high salt did not show elevation of blood pressure when compared to R rats on low salt.

TABLE 1. Group means ± SE of weekly body weights of Dahl S and R rats consuming grain-based, low (0.4% NaCl) and high (8.0% NaCl) salt diets.

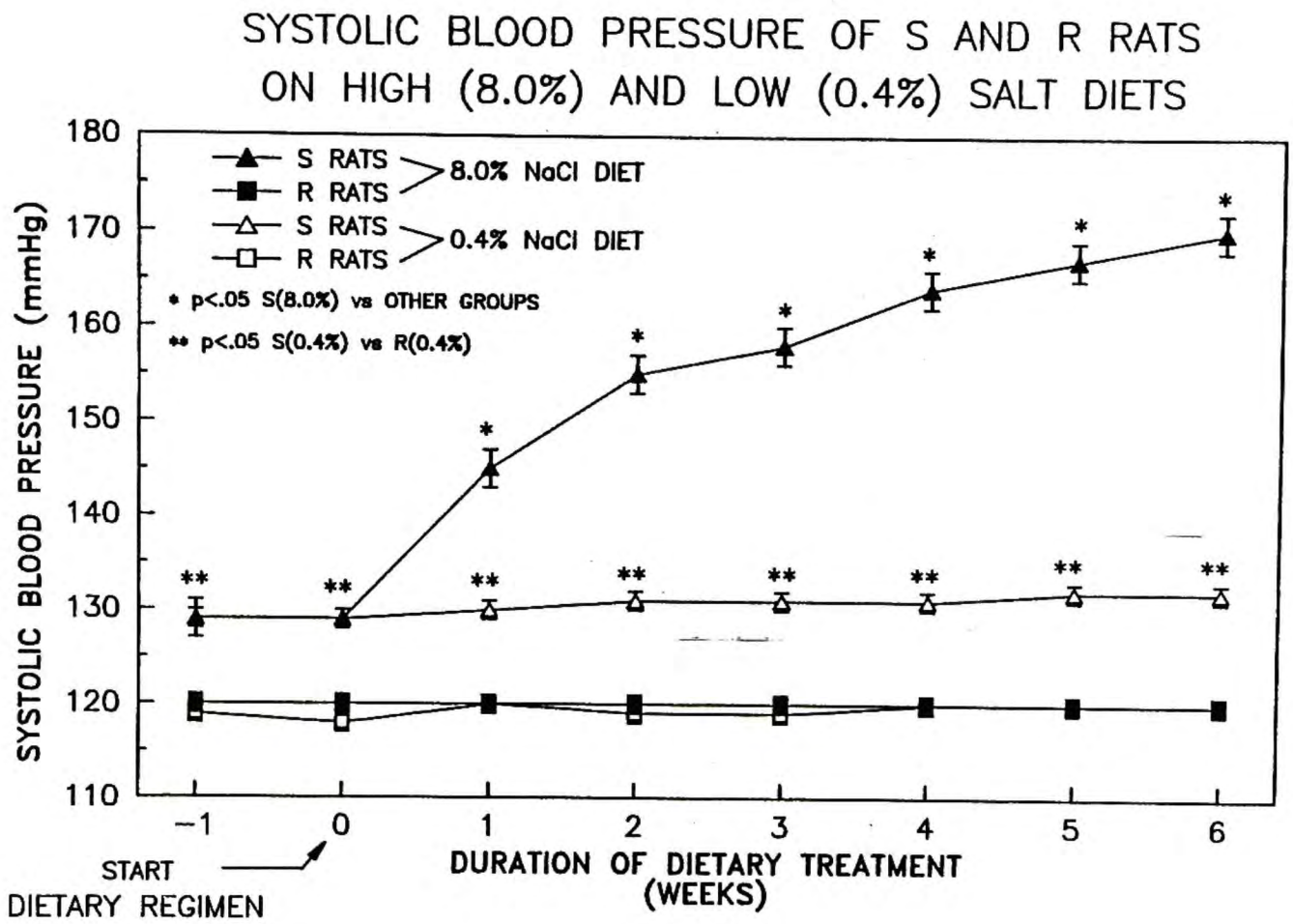
Table 1

BODY WEIGHT OF DAHL S AND R RATS CONSUMING GRAIN-BASED DIETS

	0.4% NaCl DIET		8.0% NaCl DIET		
WEEKS OF TREATMENT	R rats (N=27)	S rats (N=27)	R rats (N=33)	S rats (N=25)	p Value
-1	227 ± 9	222 ± 10	234 ± 9	232 ± 10	N.S.
0	269 ± 8	263 ± 10	274 ± 9	276 ± 11	N.S.
1	301 ± 8	296 ± 10	301 ± 7	295 ± 9	N.S.
2	328 ± 7	323 ± 7	320 ± 6	313 ± 7	N.S.
3	351 ± 6	340 ± 6	341 ± 6	336 ± 7	N.S.
4	371 ± 5	364 ± 6	361 ± 5	357 ± 7	N.S.
5	387 ± 7	382 ± 6	381 ± 5	372 ± 6	N.S.
6	409 ± 5	399 ± 5	396 ± 5	386 ± 5	N.S.

(all values in grams)

FIGURE 4. Average weekly systolic blood pressures (in mmHg) of Dahl S and R rats consuming low (0.4% NaCl) and high (8.0% NaCl) salt, grain-based diets. Observations began at seven weeks of age. All values are group mean ± SE.


SYSTOLIC BLOOD PRESSURE OF S AND R RATS
ON HIGH (8.0%) AND LOW (0.4%) SALT DIETS
S RATS
R RATS
8.0% NaCl DIET
S RATS
R RATS
0.4% NaCl DIET
* p<.05 S(8.0%) vs OTHER GROUPS
** p<.05 S(0.4%) vs R(0.4%)
SYSTOLIC BLOOD PRESSURE (mmHg)
180
170
160
150
140
130
120
110
−1
0
1
2
3
4
5
6
START
DIETARY REGIMEN
DURATION OF DIETARY TREATMENT
(WEEKS)

B. Rats consuming synthetic sodium-deficient diets.

The progressive changes in body weights for the four groups of S and R rats consuming the synthetic, high or sodium deficient diet are presented in Figure 5. Actual data values are presented in Table 3 (see APPENDIX). Body weights were significantly lower in all groups when compared to rats consuming a natural grain-based diet containing 0.4% NaCl (Table 1). In addition, while all four groups were not different in weight prior to the start of the dietary regimen, those rats (S or R) consuming the synthetic diet with 8.0% added NaCl showed significantly greater body weight after six weeks of this increased salt intake relative to rats that remained on the synthetic sodium deficient diet for the same time period. S rats consuming the 8.0% salt diet showed the greatest weight gain, significantly higher than that of R rats on the same diet.

The systolic blood pressures for the four groups of Dahl rats consuming the synthetic diet are shown in Figure 6, and in Table 4 (see APPENDIX). The progressive increase in blood pressure in the S rats on the synthetic diet (with 8.0% added salt) was similar to that of S rats on the high salt grain-based diet in our first series of experiments (Figure 4 and Table 2). Blood pressure rose to greater than 140 mmHg by the end of the first week and then increased progressively to 176 $\pm$ 1 mmHg by the sixth week of increased dietary salt intake. Blood pressures in R rats on high or low salt synthetic diets were not elevated and remained similar to those of R rats on the high or low salt grain-based diet. Both R rat groups had blood pressures of approximately 120 mmHg throughout the observation period. At the start

FIGURE 5. Average weekly body weights (grams) of Dahl S and R rats consuming synthetic, sodium-deficient (0.04% NaCl) diets, or synthetic diets with 8.0% added NaCl. Observations began at seven weeks of age. All values are group mean ± SE.

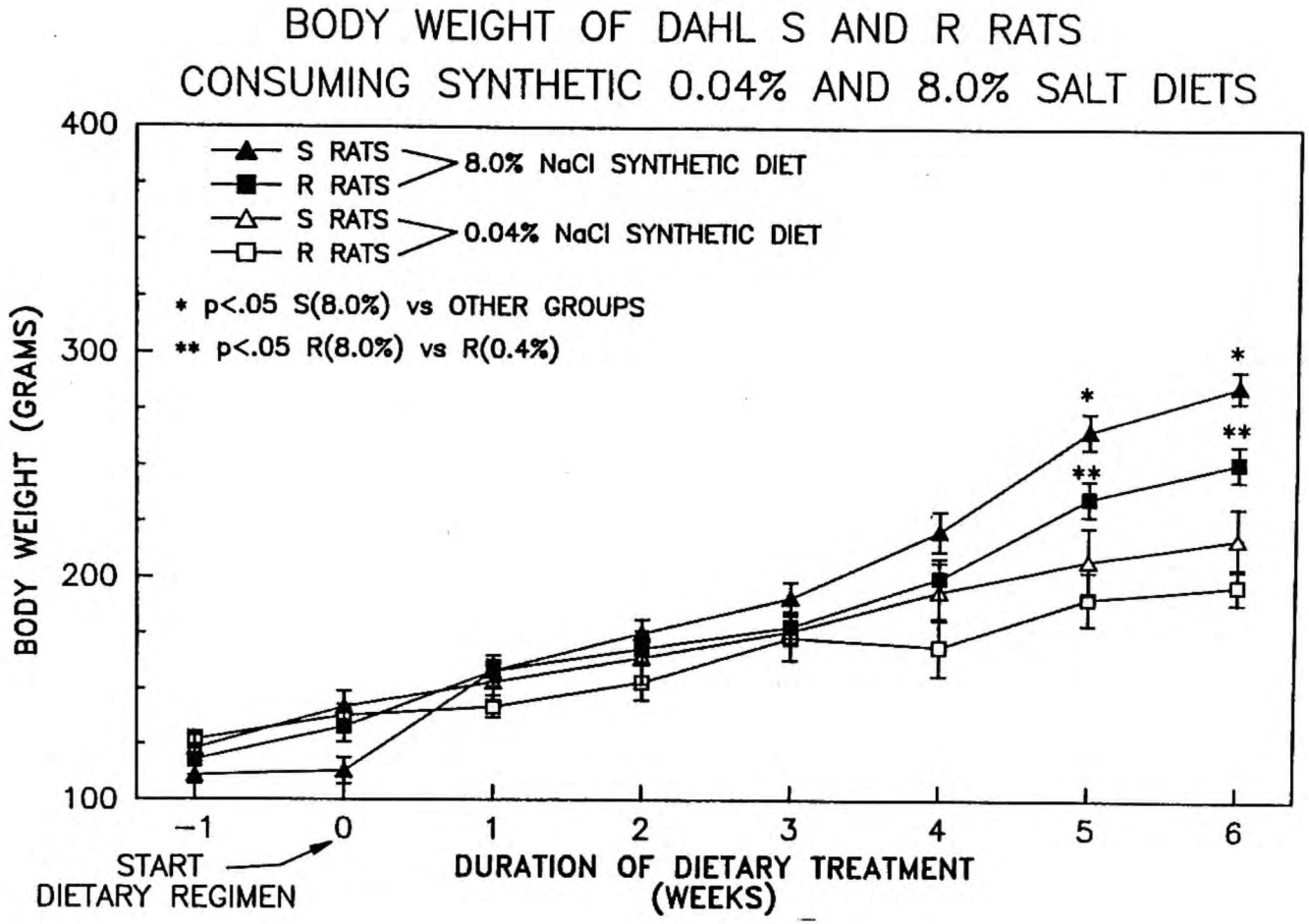
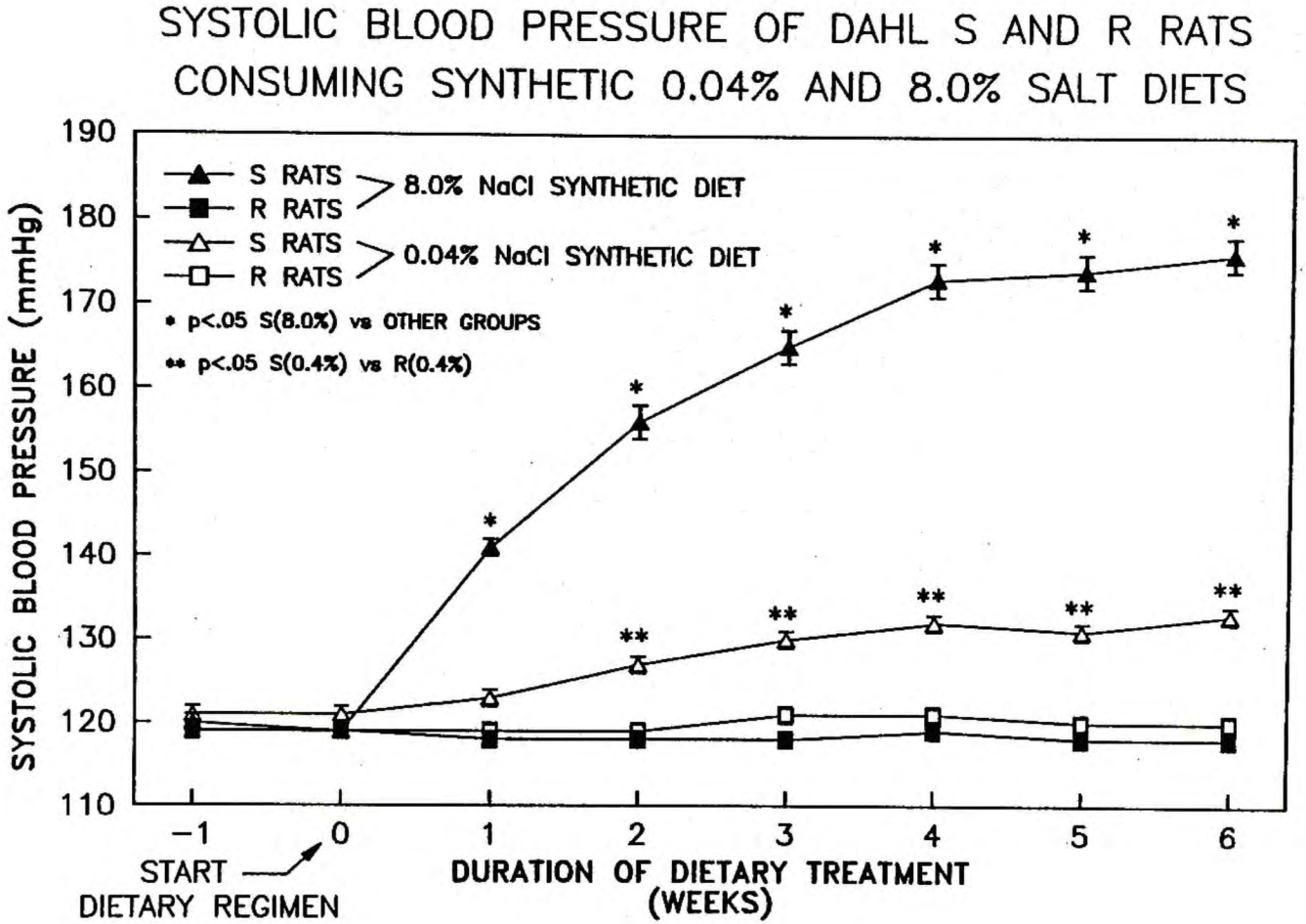

BODY WEIGHT OF DAHL S AND R RATS
CONSUMING SYNTHETIC 0.04% AND 8.0% SALT DIETS
S RATS
R RATS
8.0% NaCl SYNTHETIC DIET
S RATS
R RATS
0.04% NaCl SYNTHETIC DIET
* p<.05 S(8.0%) vs OTHER GROUPS
** p<.05 R(8.0%) vs R(0.4%)
BODY WEIGHT (GRAMS)
400
300
200
100
−1
0
1
2
3
4
5
6
START
DIETARY REGIMEN
DURATION OF DIETARY TREATMENT
(WEEKS)

FIGURE 6. Average weekly systolic blood pressures (in mmHg) of Dahl S and R rats consuming synthetic, sodium-deficient (0.04% NaCl) diets, or synthetic diets containing 8.0% added NaCl. Observations began at seven weeks of age. All values are group mean ± SE.


SYSTOLIC BLOOD PRESSURE OF DAHL S AND R RATS
CONSUMING SYNTHETIC 0.04% AND 8.0% SALT DIETS
S RATS
R RATS
8.0% NaCl SYNTHETIC DIET
S RATS
R RATS
0.04% NaCl SYNTHETIC DIET
* p<.05 S(8.0%) vs OTHER GROUPS
** p<.05 S(0.4%) vs R(0.4%)
SYSTOLIC BLOOD PRESSURE (mmHg)
190
180
170
160
150
140
130
120
110
-1
0
1
2
3
4
5
6
START
DIETARY REGIMEN
DURATION OF DIETARY TREATMENT
(WEEKS)

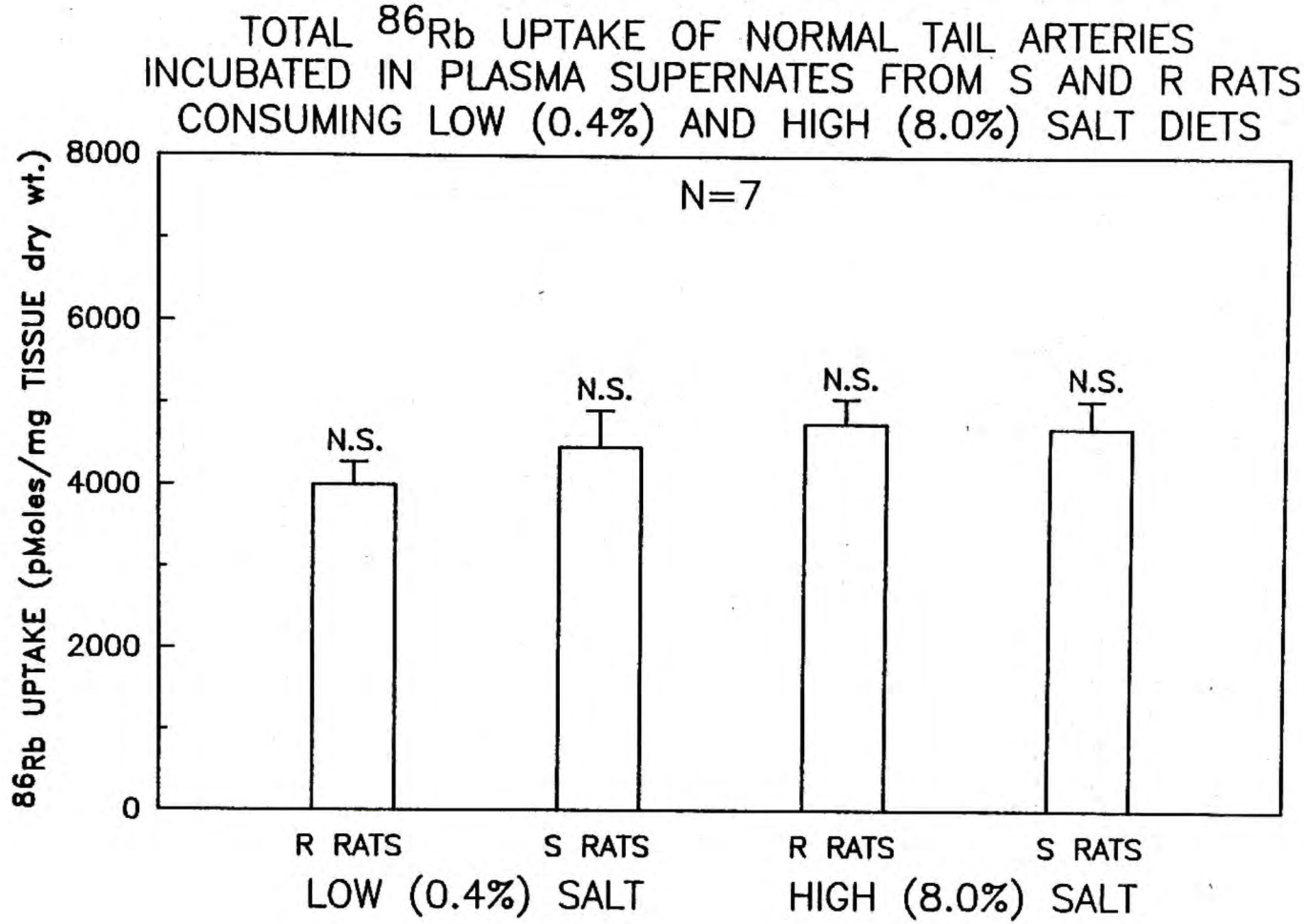
of the observations, S rats on 0.04% NaCl synthetic diets (Figure 6 and Table 4) had significantly lower blood pressure than S rats on 0.4% NaCl grain-based diets (Figure 4 and Table 2). The significantly greater blood pressure of S rats relative to R rats (129 ± 1 and 119 ± 1 mmHg), shown in Figure 4 when both were consuming low salt, was not present in S rats consuming the synthetic sodium deficient diets when the observations began (seven weeks of age). The blood pressure of these S rats did rise to 133 ± 1 mmHg by 13 weeks of age and at this time was not different from the S rats consuming the low salt grain-based diet shown in Figure 4.

II. RESULTS FROM ASSAY FOR THE PRESENCE OF OLHF IN DAHL RATS

A. ^{86}Rb uptake of tail arteries incubated in plasma supernates from Dahl rats consuming grain-based diets.

Total ^{86}Rb uptakes by tail arteries, of normal Sprague Dawley rats, incubated in boiled plasma supernates from Dahl S and R rats on low (0.4% NaCl) or high (8.0% NaCl) salt diets are presented in Figure 7. The plasma supernates from the four groups of Dahl rats all had similar effects on the uptake of ^{86}Rb. No significant differences were detected between the groups when comparing supernates from either strain when on high or low salt diets, or when comparing supernates from rats within a strain on the two different diets.

FIGURE 7. Total ^{86}Rb uptake of normal rat tail arteries incubated in boiled plasma supernates obtained from Dahl S and R rats consuming low (0.4% NaCl) and high (8.0% NaCl) salt, grain-based diets. All values are group mean ± SE.


TOTAL 86Rb UPTAKE OF NORMAL TAIL ARTERIES
INCUBATED IN PLASMA SUPERNATES FROM S AND R RATS
CONSUMING LOW (0.4%) AND HIGH (8.0%) SALT DIETS
N=7
86Rb UPTAKE (pMoles/mg TISSUE dry wt.)
8000
6000
4000
2000
0
N.S.
N.S.
N.S.
N.S.
R RATS
S RATS
R RATS
S RATS
LOW (0.4%) SALT
HIGH (8.0%) SALT

B. ^{86}Rb uptake of tail arteries incubated in supernates from Dahl rats consuming synthetic diets.

Results from the assay of boiled plasma supernates from S and R rats on the synthetic sodium deficient diet are presented in Figure 8. No significant differences in ^{86}Rb uptake were detected in normal tail arteries following incubation in plasma supernates from any of the four groups of Dahl rats on this dietary regimen. These results are similar to those obtained from Dahl rats on the grain-based diets shown in Figure 7.

C. Plasma supernate composition.

Chemical composition of the boiled plasma supernates from S or R rats consuming the natural grain-based diet are presented in Table 5. Electrolytes, osmolality, creatinine and blood urea nitrogen (BUN) were not significantly different between the four groups. Protein levels were however significantly increased in supernates from hypertensive S rats on the high salt diet relative to S rats on low salt or R rats on either diet.

Supernate compositions for the Dahl S and R rats consuming the synthetic diet are shown in Table 6. Similar to our findings with the grain-based diet (Table 5), no significant differences in electrolytes, osmolality, creatinine or BUN were observed. Again, in this study as well, the protein levels were significantly higher in S rats on 8.0% NaCl relative to the other three groups.

FIGURE 8. Total ^{86}Rb uptake of normal rat tail arteries incubated in boiled plasma supernates obtained from Dahl S and R rats consuming synthetic, sodium-deficient (0.04% NaCl) diets, or synthetic diets containing 8.0% added NaCl. All values are group mean $\pm$ SE.

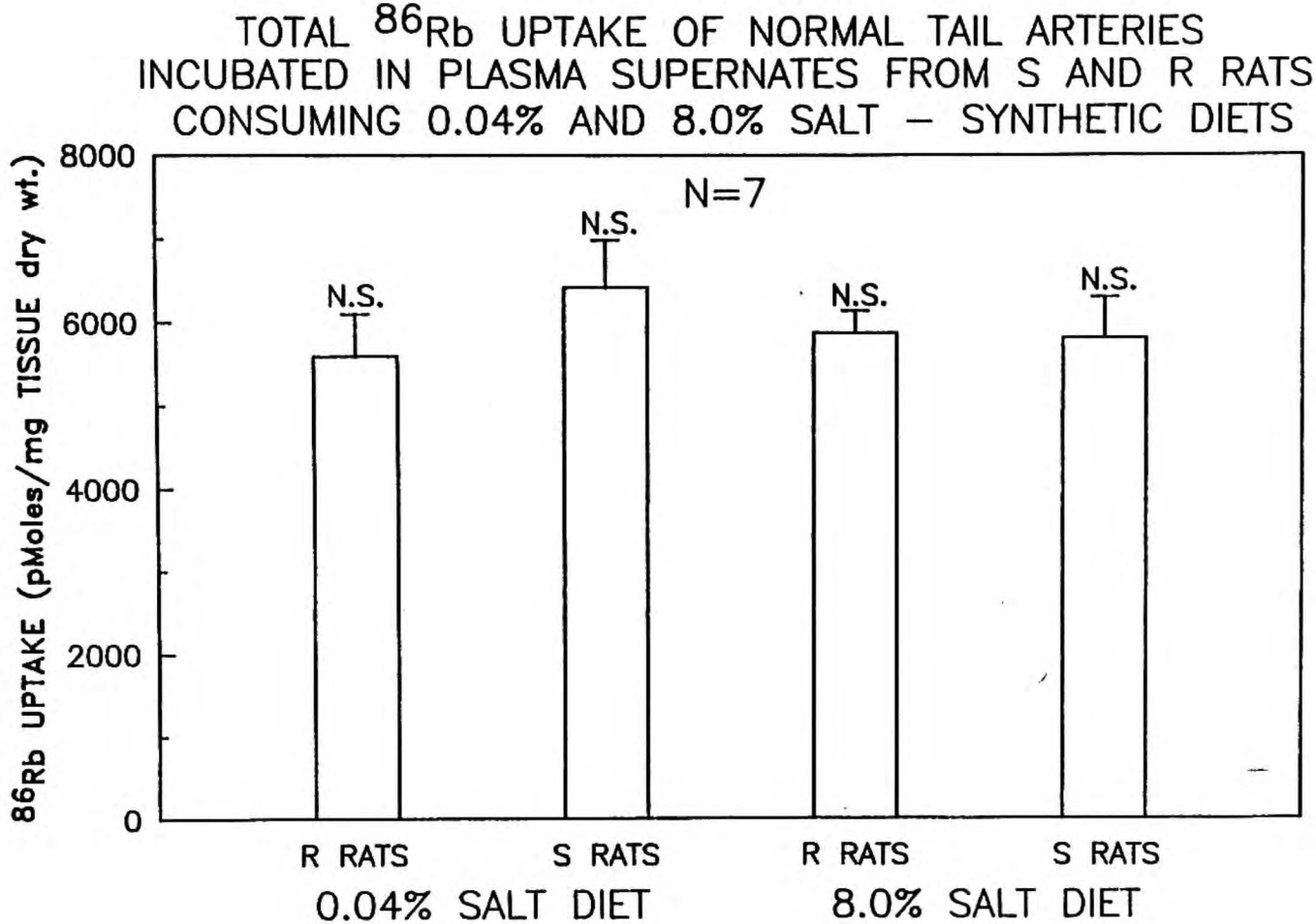

TOTAL 86Rb UPTAKE OF NORMAL TAIL ARTERIES
INCUBATED IN PLASMA SUPERNATES FROM S AND R RATS
CONSUMING 0.04% AND 8.0% SALT – SYNTHETIC DIETS
N=7
86Rb UPTAKE (pMoles/mg TISSUE dry wt.)
8000
6000
4000
2000
0
N.S.
N.S.
N.S.
N.S.
R RATS
S RATS
R RATS
S RATS
0.04% SALT DIET
8.0% SALT DIET

TABLE 5. Composition of boiled plasma supernates from Dahl S and R rats consuming low (0.4% NaCl) or high (8.0% NaCl) salt, grain-based diets. All values are group mean ± SE.

Table 5

COMPOSITION OF PLASMA SUPERNATES FROM DAHL S AND R RATS CONSUMING GRAIN-BASED DIETS

	0.4% NaCl DIET		8.0% NaCl DIET		
	R rats	S rats	R rats	S rats	p Value
Na^+ (mEq/L) N=7	145.7 ± 0.57	146.5 ± 0.51	146.9 ± 1.13	147.1 ± 0.40	N.S.
K^+ (mEq/L) N=7	3.89 ± 0.12	3.99 ± 0.07	3.79 ± 0.07	3.92 ± 0.09	N.S.
Cl^- (mEq/L) N=7	109.8 ± 1.01	110.0 ± 0.98	111.2 ± 1.14	110.6 ± 0.97	N.S.
Ca^{++} (mEq/L) N=7	2.21 ± 0.11	2.37 ± 0.10	2.31 ± 0.09	2.27 ± 0.07	N.S.
Mg^{++} (mEq/L) N=7	0.94 ± 0.05	0.96 ± 0.04	0.96 ± 0.03	0.94 ± 0.04	N.S.
Osmolality (mOsm) N=5	311.3 ± 2.3	311.5 ± 3.4	307.8 ± 3.5	311.5 ± 4.1	N.S.
Creatinine (mg%) N=7	0.85 ± 0.05	0.84 ± 0.07	0.80 ± 0.09	0.84 ± 0.09	N.S.
BUN (mg%) N=7	20.50 ± 1.11	22.01 ± 1.11	23.66 ± 1.67	22.78 ± 1.28	N.S.
Protein (mg%) N=6	430.0 ± 33.8	445.5 ± 20.0	440.1 ± 18.1	568.8* ± 37.1	$p<.05$

TABLE 6. Composition of boiled plasma supernates from Dahl S and R rats consuming synthetic sodium-deficient (0.04% NaCl) diets, or synthetic diets containing 8.0% added NaCl. All values are group mean ± SE.

Table 6

COMPOSITION OF PLASMA SUPERNATES FROM DAHL S AND R RATS CONSUMING SYNTHETIC SODIUM-DEFICIENT DIETS

	0.04% NaCl DIET		8.0% NaCl DIET		
	R rats	S rats	R rats	S rats	p Value
Na^+ (mEq/L) N=7	147.4 ± 0.7	148.0 ± 0.9	144.6 ± 0.4	145.6 ± 0.5	N.S.
K^+ (mEq/L) N=7	3.59 ± 0.10	3.59 ± 0.13	4.00 ± 0.14	4.13 ± 0.16	N.S.
Cl^- (mEq/L) N=7	111.1 ± 1.0	112.9 ± 1.5	110.7 ± 1.2	110.9 ± 0.5	N.S.
Ca^{++} (mEq/L) N=7	1.26 ± 0.06	1.29 ± 0.13	1.44 ± 0.17	1.47 ± 0.21	N.S.
Mg^{++} (mEq/L) N=7	0.88 ± 0.04	0.90 ± 0.04	0.86 ± 0.02	0.86 ± 0.06	N.S.
Osmolality (mOsm) N=7	328.8 ± 16.0	324.7 ± 8.0	329.4 ± 17.3	321.3 ± 5.3	N.S.
Creatinine (mg%) N=4	0.57 ± 0.02	0.56 ± 0.06	0.61 ± 0.02	0.60 ± 0.05	N.S.
BUN (mg%) N=5	21.43 ± 3.34	22.80 ± 1.11	25.76 ± 0.70	25.53 ± 3.55	N.S.
Protein (mg%) N=4	366.29 ± 17.36	404.50 ± 39.66	391.01 ± 15.35	528.84* ± 24.93	$p<.05$

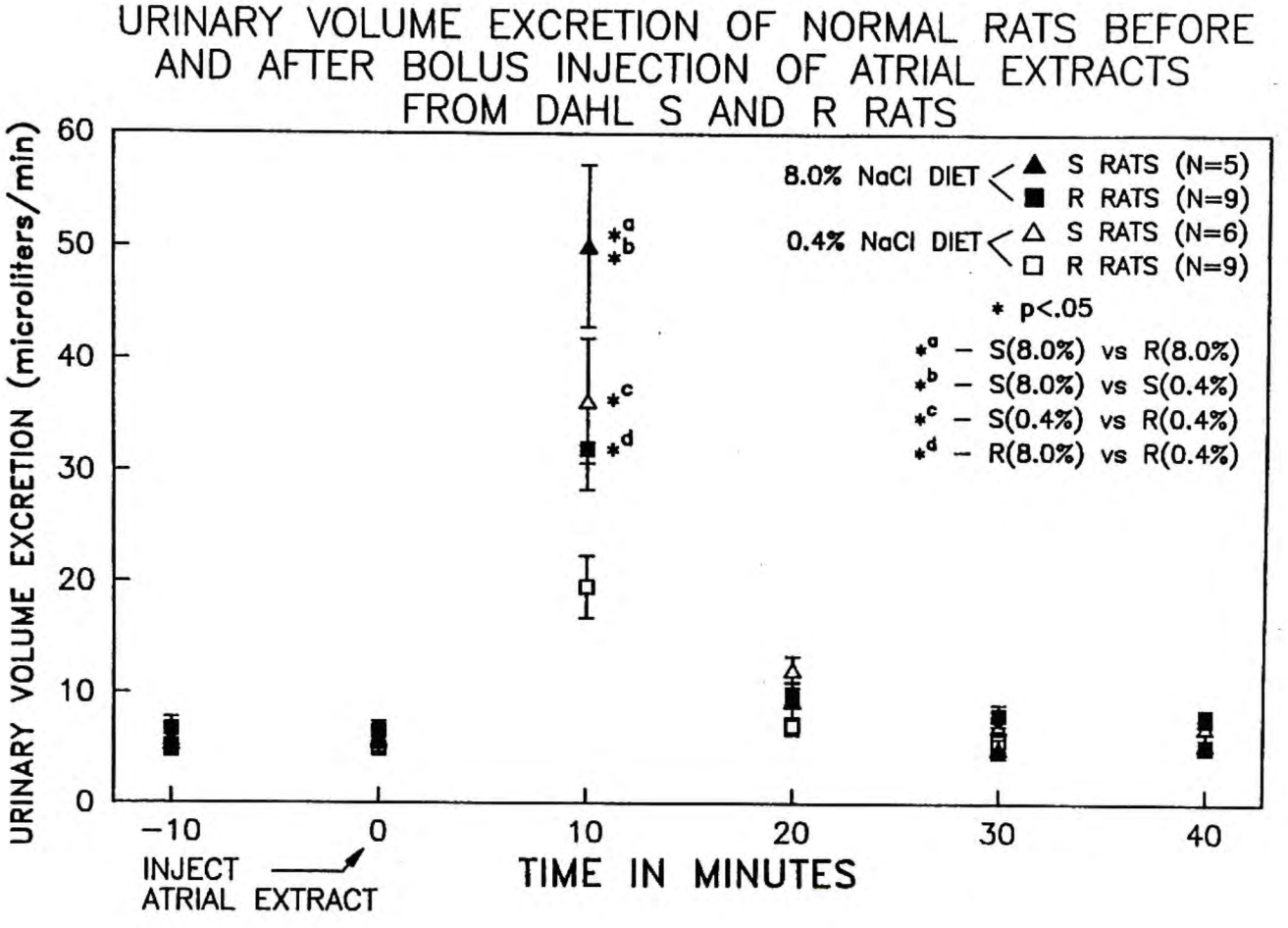
III. DATA FROM ANF STUDY

A. Assay for atrial levels of ANF in Dahl S and R rats.

1. Differences in levels of atrial ANF as determined by natriuresis and diuresis in normal rats.

The effects of i.v. injection of atrial extracts, prepared from Dahl S or R rats on low (0.4% NaCl) or high (8.0% NaCl) salt diets, on urine volume in normal Sprague Dawley assay rats are presented in Figure 9. Prior to injection of the extract, the urine production of the four groups of normal assay rats were not significantly different. Ten minutes following injection, atrial extracts from all four groups of Dahl rats produced significant diuresis in these normal assay rats. Bioassay rats receiving injection of atrial extracts from S rats on high salt showed the greatest response which was significantly larger than the response to atrial extracts from S rats on low salt or R rats on high salt (48.9 ± 8.6 vs. 36.3 ± 5.8 and 31.9 ± 3.7 microliters/min respectively). Extracts from S rats on low salt produced greater responses than extracts from R rats on low salt (36.3 ± 5.8 vs. 19.5 ± 2.8), and responses were greater following extracts from R rats on high salt relative to R rats on low salt (31.9 ± 3.7 vs. 19.5 ± 2.8). These differences were not significant after 20 minutes, and at 30 minutes the urine excretion of the four groups of assay rats was not different from control pre-injection levels. Ventricular extracts were not used in these studies, as de Bold et al. (1981) had demonstrated that they do not have this diuretic activity.

FIGURE 9. Urinary volume excretion (UV) of normal Sprague Dawley bioassay rats prior to, and following i.v. injection of 0.20 ml of atrial extract obtained from Dahl S and R rats consuming low (0.4% NaCl) and high (8.0% NaCl) salt diets. All values are group mean ± SE.


URINARY VOLUME EXCRETION OF NORMAL RATS BEFORE AND AFTER BOLUS INJECTION OF ATRIAL EXTRACTS FROM DAHL S AND R RATS
URINARY VOLUME EXCRETION (microliters/min)
60
50
40
30
20
10
0
8.0% NaCl DIET
S RATS (N=5)
R RATS (N=9)
0.4% NaCl DIET
S RATS (N=6)
R RATS (N=9)
* p<.05
*a – S(8.0%) vs R(8.0%)
*b – S(8.0%) vs S(0.4%)
*c – S(0.4%) vs R(0.4%)
*d – R(8.0%) vs R(0.4%)
*a
*b
*c
*d
−10
0
10
20
30
40
INJECT ATRIAL EXTRACT
TIME IN MINUTES

The sodium excretion, before and after bolus injection of atrial extract, of these same Sprague Dawley assay rats is presented in Figure 10. These results are similar to those for urine excretion. Sodium excretion ($U_{Na}V$) was not different among the four groups of assay rats prior to injection. Ten minutes following injection, extracts from S rats on high salt produced the greatest response, significantly greater than the response to extracts from S rats on low salt or R rats on high salt (6.5 ± 1.4 vs. 4.8 ± 0.9 and 3.3 ± 0.6 microequivalents/min respectively). Additionally, greater responses were seen following injection of extracts from rats on high salt relative to low salt in both strains (6.5 ± 1.4 vs. 4.8 ± 0.9 for S rats, and 3.3 ± 0.6 vs. 1.7 ± 0.5 for R rats). The differences were not significant at 20 minutes and were, again, not different from control by 30 minutes following injection.

2. Differences in atrial content of ANF as determined by radioimmunoassay of Dahl rat atrial extracts.

Data from the determination of atrial levels of immunoreactive AP III by radioimmunoassay is presented in Figures 11 and 12. Figure 11 represents the comparison of atrial extracts between S and R rat strains when both consumed low or high salt diets. No significant difference was detected between S and R strains when on the low salt intake. However, when the rats were consuming the high salt diets, the extracts from hypertensive S rats had 50% greater immunoreactive levels of AP III than R rats on the same diet.

FIGURE 10. Urinary sodium excretion ($U_{Na}V$) of normal Sprague Dawley bioassay rats prior to, and following i.v. injection of 0.20 ml of atrial extract obtained from Dahl S and R rats consuming low (0.4% NaCl) and high (8.0% NaCl) salt diets. All values are group mean ± SE.

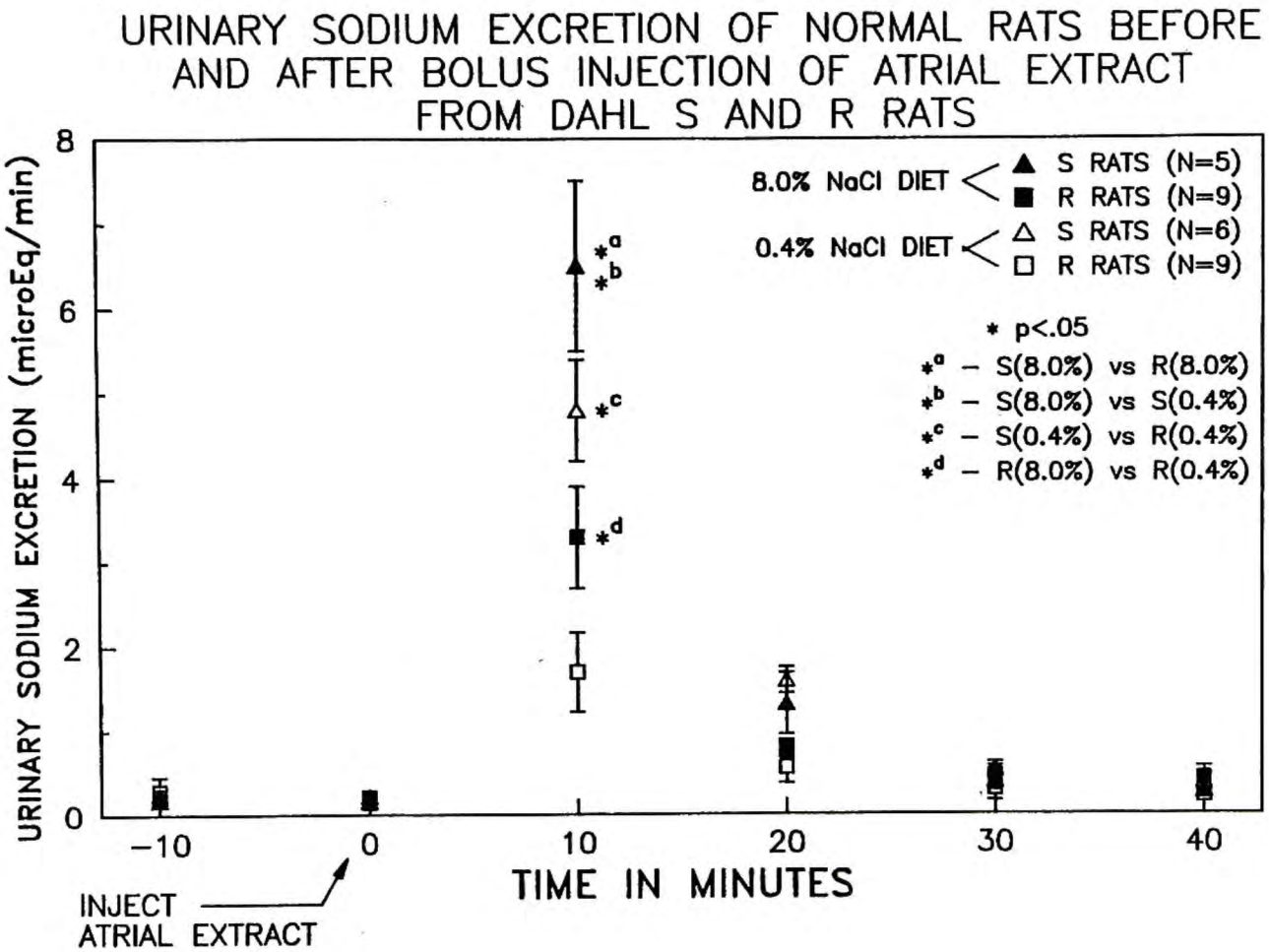

URINARY SODIUM EXCRETION OF NORMAL RATS BEFORE AND AFTER BOLUS INJECTION OF ATRIAL EXTRACT FROM DAHL S AND R RATS
URINARY SODIUM EXCRETION (microEq/min)
8
6
4
2
0
8.0% NaCl DIET
S RATS (N=5)
R RATS (N=9)
0.4% NaCl DIET
S RATS (N=6)
R RATS (N=9)
* p<.05
*a – S(8.0%) vs R(8.0%)
*b – S(8.0%) vs S(0.4%)
*c – S(0.4%) vs R(0.4%)
*d – R(8.0%) vs R(0.4%)
*a
*b
*c
*d
−10
0
10
20
30
40
TIME IN MINUTES
INJECT ATRIAL EXTRACT

FIGURE 11. Immunoreactive AP III content of atrial extracts obtained from Dahl S relative to Dahl R rats when consuming low (0.4% NaCl) or high (8.0% NaCl) salt diets. All values are group mean ± SE.

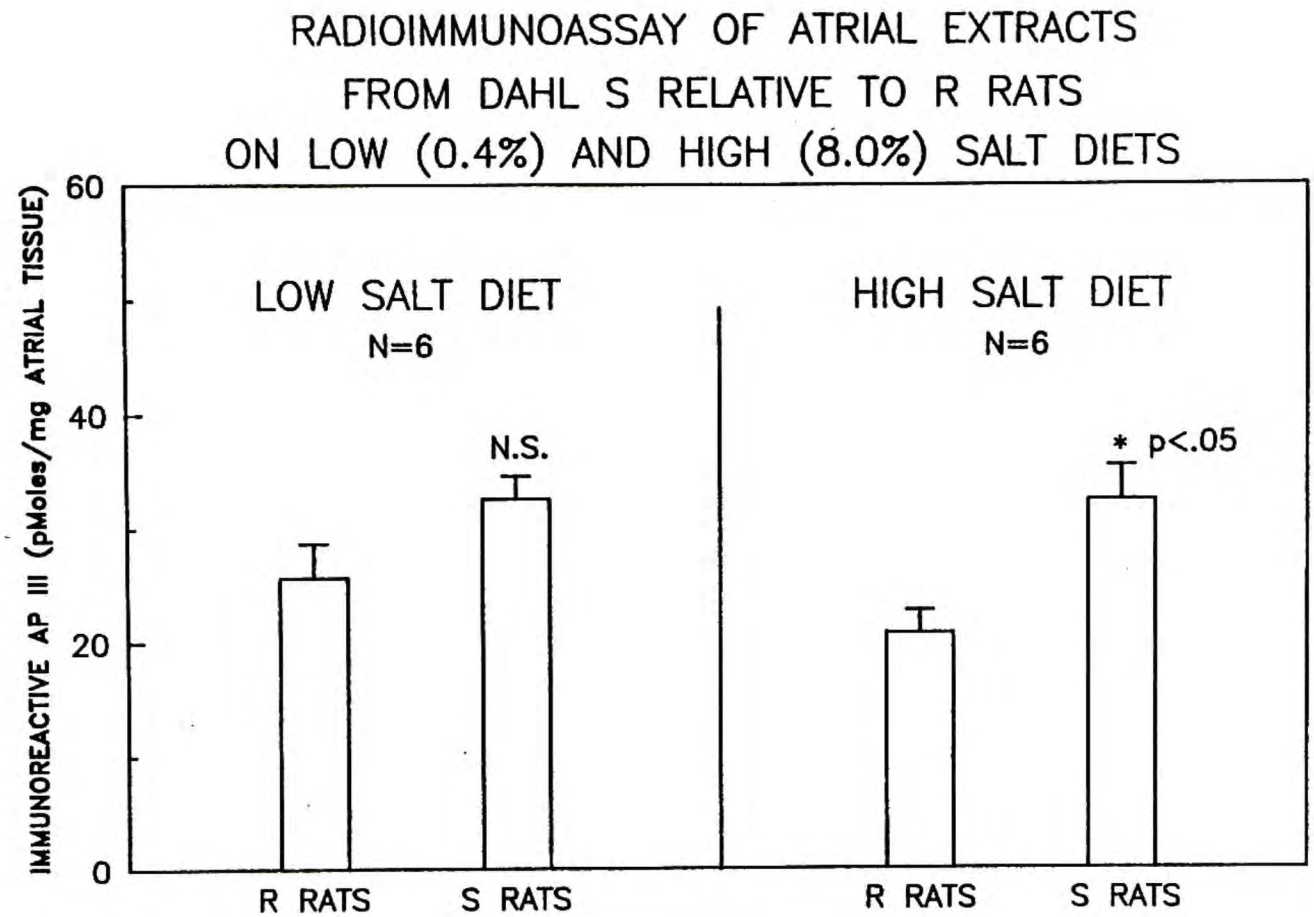

RADIOIMMUNOASSAY OF ATRIAL EXTRACTS
FROM DAHL S RELATIVE TO R RATS
ON LOW (0.4%) AND HIGH (8.0%) SALT DIETS
IMMUNOREACTIVE AP III (pMoles/mg ATRIAL TISSUE)
60
40
20
0
LOW SALT DIET
N=6
N.S.
HIGH SALT DIET
N=6
* p<.05
R RATS
S RATS
R RATS
S RATS

Figure 12 represents the comparison of extracts from rats within each of the S or R strains. The data is from the same study shown in Figure 11. Atrial levels of immunoreactive AP III were not significantly different in R rat extracts when comparing rats on low relative to high salt diets, or in extracts from S rats when the same comparison is made.

3. Bioassay for ANF by effects on aortic contractile responses to norepinephrine.

Figure 13 represents the average dose-responses curves to norepinephrine of normal rat aortic rings in the presence of various concentrations of atrial extract. The control response to norepinephrine was dose-dependent as expected. Threshold responses were observed at 3×10^{-10} M norepinephrine concentrations. Maximal responses were present at a concentration of 10^{-5} M. The EC_{50} for the response to norepinephrine was 10.2 ± 1.1 nM (N=27). The progressive right hand shift in the norepinephrine dose-response curve following increasing concentration of atrial extract in the bath is significant at a concentration of atrial extract equivalent to 20 micrograms per ml. The maximal tension development in response to norepinephrine was affected significantly only at the highest concentration (160 micrograms per ml) of atrial extract.

The average EC_{50} values for norepinephrine-induced contractions, of the dose-response curves presented in Figure 13, are shown in Figure 14 plotted against the concentration of atrial extract used. The actual EC_{50} values are presented in Table 7 along with EC_{50} values for similarly treated aortic rings in the presence of two concentrations of

FIGURE 12. Immunoreactive AP III content of atrial extracts from Dahl S or R rats when consuming high (8.0% NaCl) relative to low (0.4% NaCl) salt diets. All values are group mean ± SE. This data is the same as that presented in Figure 11.

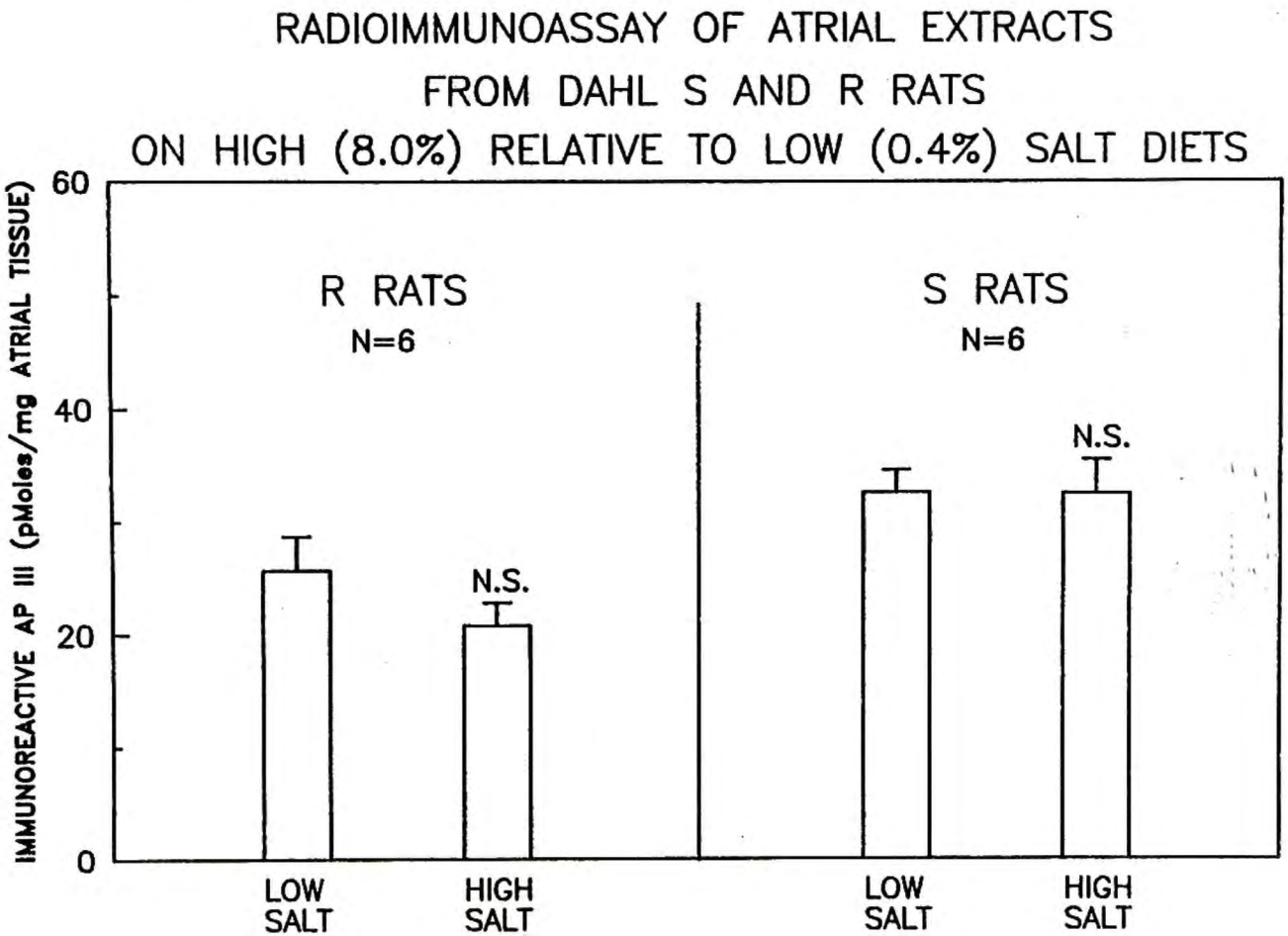

RADIOIMMUNOASSAY OF ATRIAL EXTRACTS
FROM DAHL S AND R RATS
ON HIGH (8.0%) RELATIVE TO LOW (0.4%) SALT DIETS
IMMUNOREACTIVE AP III (pMoles/mg ATRIAL TISSUE)
60
40
20
0
R RATS
N=6
S RATS
N=6
N.S.
N.S.
LOW SALT
HIGH SALT
LOW SALT
HIGH SALT

FIGURE 13. Average dose-response curves for norepinephrine in normal rat aortic rings exposed to various concentrations of atrial extract. Extracts were pooled from a number of normal Sprague Dawley rats. All values are group mean ± SE.

DOSE–RESPONSE CURVES FOR NOREPINEPHRINE IN NORMAL RAT AORTIC RINGS EXPOSED TO VARIOUS CONCENTRATIONS OF ATRIAL EXTRACT

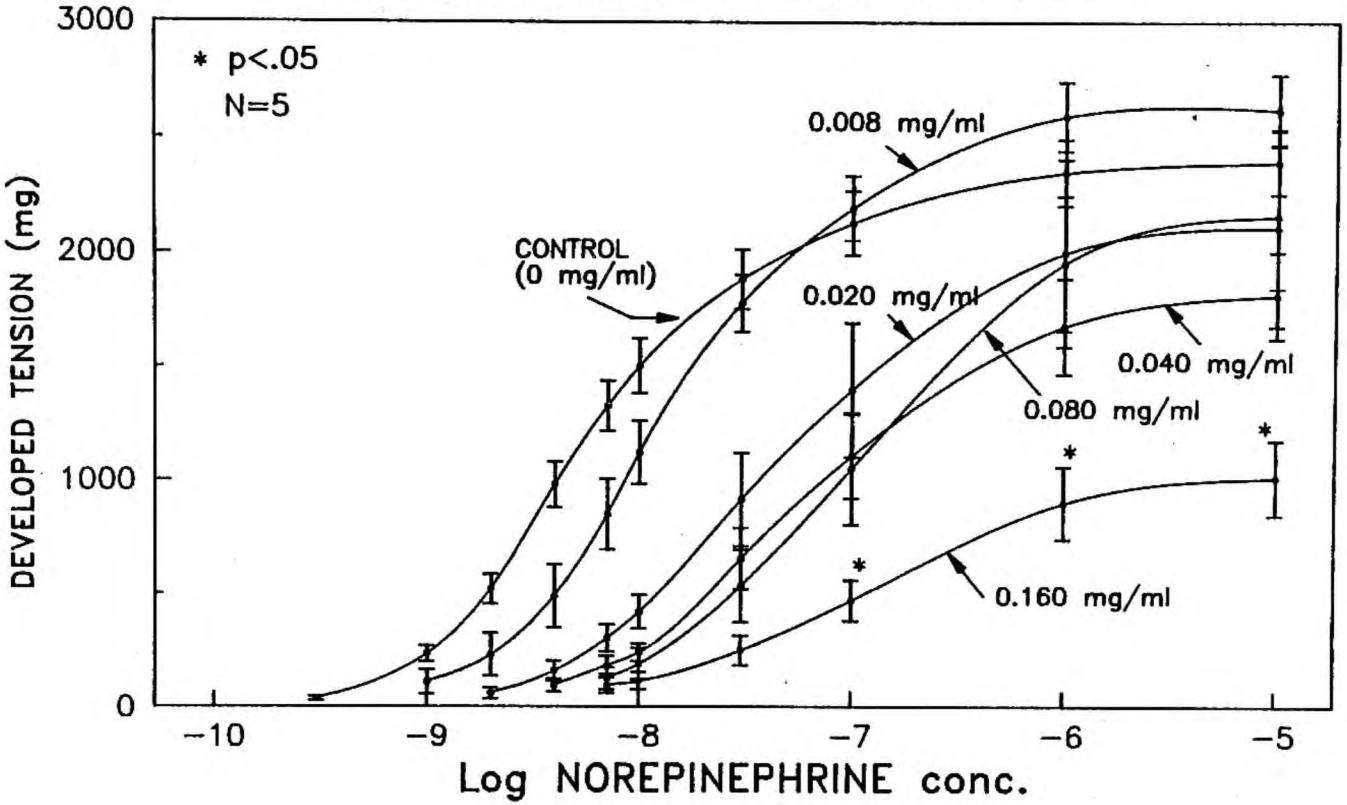


FIGURE 14. Average EC_{50} values for norepinephrine-induced contractions of normal Sprague Dawley rat aortic rings plotted against the bath concentration of atrial extract. All values are group mean ± SE. The starred values indicate significant differences from control.

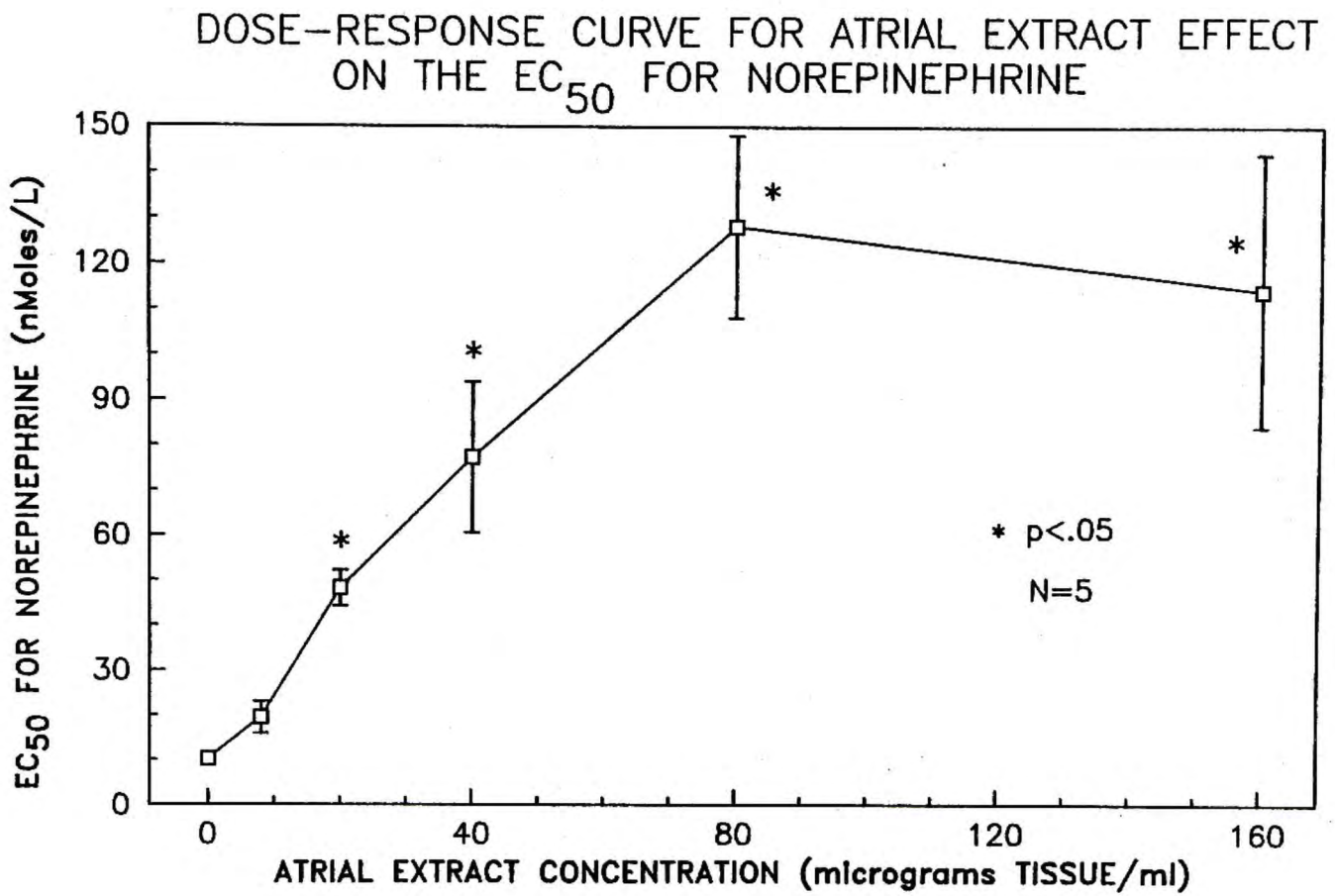

DOSE–RESPONSE CURVE FOR ATRIAL EXTRACT EFFECT ON THE EC_{50} FOR NOREPINEPHRINE
EC_{50} FOR NOREPINEPHRINE (nMoles/L)
150
120
90
60
30
0
0
40
80
120
160
ATRIAL EXTRACT CONCENTRATION (micrograms TISSUE/ml)
* p<.05
N=5

TABLE 7. Group means ± SE of ECP_{50} values, for norepinephrine-induced contractions, of normal rat aortic rings before and after exposure to various concentrations of atrial or ventricular tissue extracts. All values are group mean ± SE.

Table 7

AVERAGE EC_{50} VALUES FOR NOREPINEPHRINE IN AORTIC RINGS BEFORE AND AFTER EXPOSURE TO ATRIAL OR VENTRICULAR TISSUE EXTRACTS

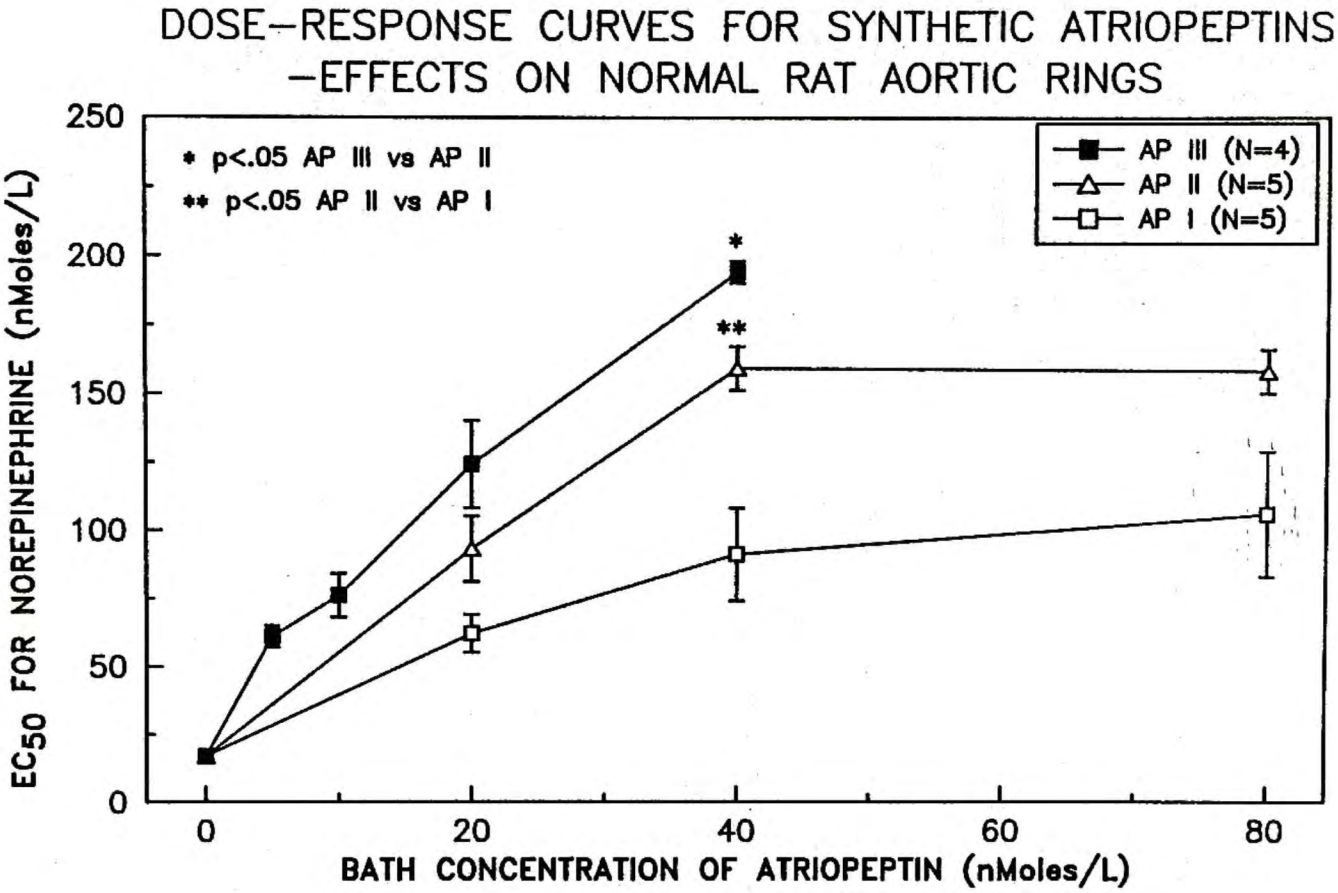
BATH CONCENTRATION OF EXTRACT (micrograms/ml)	EC_{50} FOR NOREPINEPHRINE (nMoles/Liter) BEFORE ADDITION OF EXTRACT	AFTER ADDITION OF EXTRACT	p Value
Atrial Extract			
8	9.71 ± 2.04	19.54 ± 3.55	p<.05 N=6
20	9.52 ± 2.89	48.21 ± 4.04	p<.05 N=5
40	9.49 ± 2.67	77.23 ± 16.72	p<.05 N=6
80	11.99 ± 3.78	128.34 ± 21.68	p<.05 N=5
160	10.73 ± 1.63	113.58 ± 29.62	p<.05 N=5
Ventricular Extract	BEFORE	AFTER	
40	13.12 ± 1.66	12.70 ± 1.60	N.S. N=4
160	14.18 ± 2.16	13.37 ± 1.05	N.S. N=4

ventricular extracts. As can be seen, atrial extracts produced dose-dependent increases in the EC_{50} for norepinephrine. The maximal effect of atrial extracts was seen at a concentration of 80 micrograms per ml. Ventricular extracts did not produce this effect on the response to norepinephrine.

Analysis of this dose response curve using the Hill plot gave an EC_{50} for the atrial extract effect corresponding to 41 micrograms per ml. Similar analysis by the Woolf plot (S/V vs. S) provided an EC_{50} value of 38 micrograms per ml for the effect. To maximize any apparent differences in content of ANF, concentrations of atrial tissue corresponding to 40 micrograms per ml were used in comparing atrial extracts from Dahl S and R rats for ANF content. This concentration was achieved by adding 20 microliters of atrial extract from Dahl rats (for preparation details see METHODS) to the 25 ml capacity contractility bath. Concentrations greater than this would cause a near maximal or maximal effect which would obscure any difference in ANF content between extracts.

Figure 15 shows the dose response curves for the three synthetic atriopeptins AP I, AP II and AP III for their effect on the EC_{50} for norepinephrine-induced contractions of normal rat aortic rings. AP III caused the greatest increase in EC_{50} for norepinephrine, followed by AP II, and AP I to a lesser extent. At bath concentrations of 40 nMoles/liter, AP III was twice as potent as AP I in this effect. Thus, the effect of atrial extracts shown in Figures 13 and 14 is similar to the effects of these highly pure synthetic forms of ANF.

FIGURE 15. Average EC_{50} values for norepinephrine-induced contractions of normal rat aortic rings plotted against the bath concentration of three synthetic atriopeptins (AP I, AP II and AP III). All values are group mean $\pm$ SE.


DOSE–RESPONSE CURVES FOR SYNTHETIC ATRIOPEPTINS
–EFFECTS ON NORMAL RAT AORTIC RINGS
* p<.05 AP III vs AP II
** p<.05 AP II vs AP I
AP III (N=4)
AP II (N=5)
AP I (N=5)
EC50 FOR NOREPINEPHRINE (nMoles/L)
BATH CONCENTRATION OF ATRIOPEPTIN (nMoles/L)
0
50
100
150
200
250
0
20
40
60
80
*
**

Figures 16 through 19 represent several comparisons of the EC_{50} values for norepinephrine of normal rat aortic rings prior to, and following the addition of atrial extract from Dahl S or R rats, to the organ bath (40 micrograms per ml final concentration). Figure 16 represents the comparison of S and R rats when both consumed the low salt diets (0.4% NaCl) for six weeks. Prior to addition of the extract, the aortic rings were not different in their responsiveness to norepinephrine (EC_{50} values 14.2 ± 2.4 and 15.7 ± 3.2 nM for R and S rats respectively). Ten minutes following addition of the extracts, rings treated with extracts from S rats showed significantly greater EC_{50} values (were less responsive) than aortic rings treated with extracts from R rats (93.5 ± 9.7 vs. 69.2 ± 10.5 nM), suggesting higher levels of ANF-like activity in extracts from S relative to R rats when both were normotensive and consuming low salt diets.

Figure 17 represents the comparison between S and R rats when both consumed high salt (8.0% NaCl) diets. Again, prior to treatment with the extract, the normal aortic rings were not different in their responses to norepinephrine (EC_{50} values 14.2 ± 3.7 and 14.8 ± 3.6 nM for R and S rats respectively). Ten minutes following addition of the atrial extract, rings treated with atrial extracts from S rats had significantly higher EC_{50} values than normal aortic rings treated with atrial extracts from R rats (75.1 ± 11.8 vs. 50.2 ± 7.4 nM respectively), again demonstrating greater content of ANF in atrial extracts from S relative to R rats.

FIGURE 16. Comparison of EC_{50} values for norepinephrine-induced contractions, of normal rat aortic rings prior to, and following application of atrial extract from Dahl S or R rats consuming low (0.4% NaCl) salt diets. Final bath concentration of extract was 40 micrograms atrial tissue per ml. All values are group mean ± SE.

EFFECTS OF ATRIAL EXTRACTS FROM S AND R RATS ON LOW (0.4%) SALT ON THE EC_{50} FOR NOREPINEPHRINE IN NORMAL RAT AORTIC RINGS

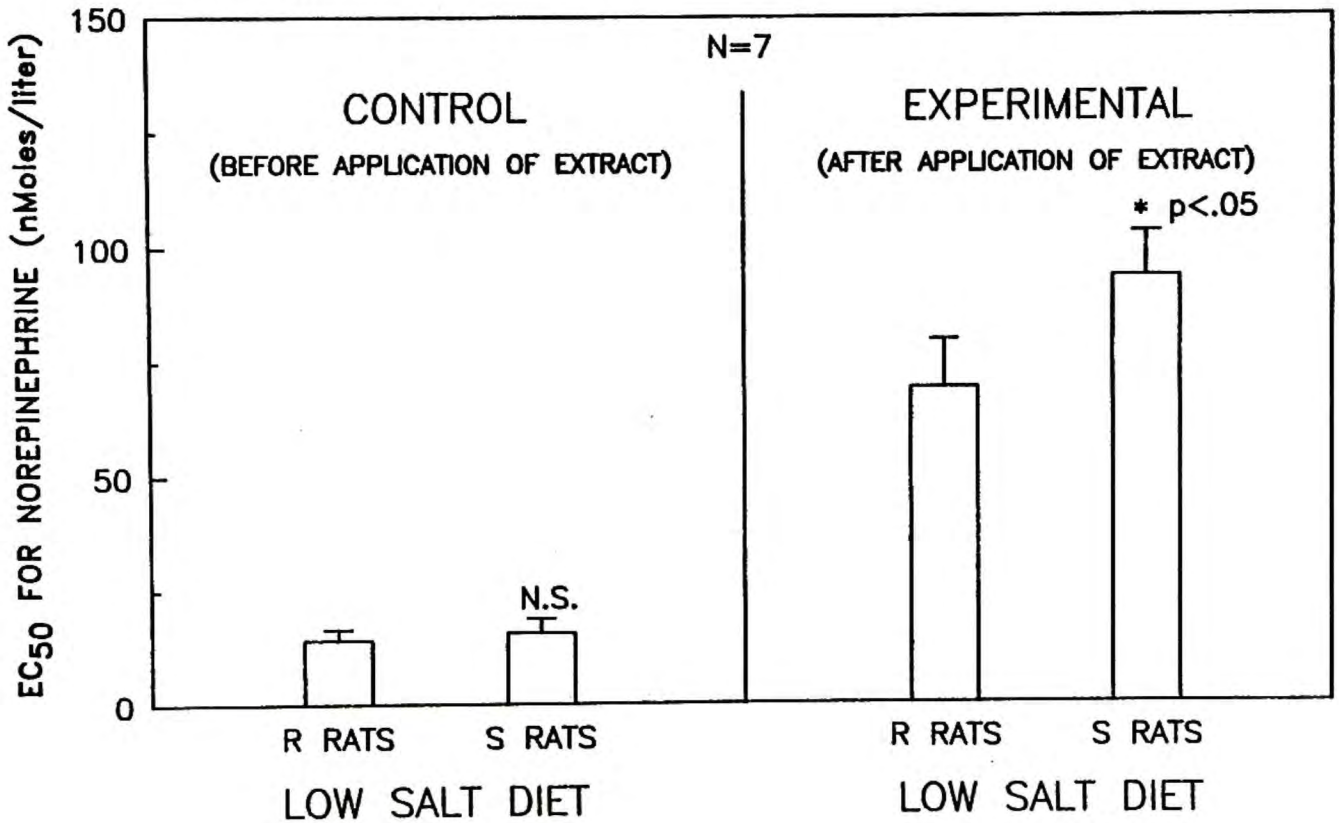
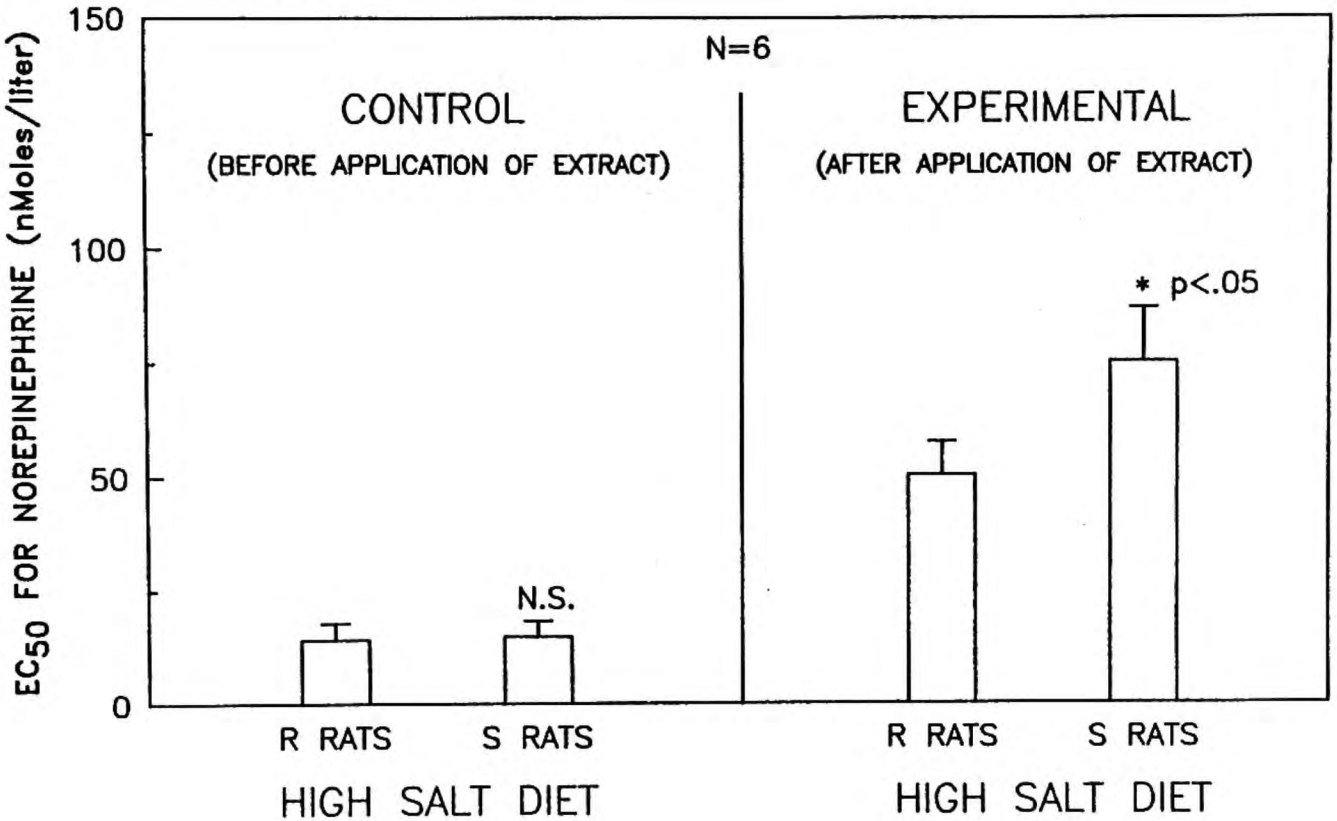


FIGURE 17. Comparison of EC_{50} values for norepinephrine-induced contractions, of normal rat aortic rings prior to, and following application of atrial extracts from Dahl S or R rats consuming high (8.0% NaCl) salt diets. Final bath concentration of extract was 40 micrograms atrial tissue per ml. All values are group mean ± SE.

EFFECTS OF ATRIAL EXTRACTS FROM S AND R RATS ON HIGH (8.0%) SALT ON THE EC_{50} FOR NOREPINEPHRINE IN NORMAL RAT AORTIC RINGS



Figures 18 and 19 represent comparisons within strains for differences in atrial ANF content due to high or low salt intake. Figure 18 shows the responses of normal rings to extracts from R rats on low or high salt. Responses were not significantly different (14.2 ± 2.4 and 14.2 ± 3.7 nM for R rats on low and high salt respectively) prior to, or following the addition of atrial extract (69.2 ± 10.5 and 50.2 ± 7.4 nM respectively), suggesting that there is no difference between atrial extracts of R rats due to salt intake alone.

Similar results were obtained in comparing responses to extracts from rats on low relative to high salt diets in S rat atrial extracts as shown in Figure 19. The EC_{50} values of the normal aortic rings were not different (15.7 ± 3.3 and 14.8 ± 3.6 nM for S rats on low and high salt respectively) prior to or following addition of the atrial extracts (93.5 ± 9.7 vs. 75.1 ± 11.8 nM).

B. Responsiveness of Dahl S and R rats to atrial extracts.

1. Differences in the natriuretic and diuretic response of Dahl rats to i.v. injection of atrial extracts.

Figure 20 represents the urinary volume excretion of normotensive Dahl S and R rats in response to the bolus i.v. injection of 0.20 ml of atrial extract from normal Sprague Dawley rats. The two control urine volume collections (-10 and 0 minutes), prior to atrial extract injection, of the S or R assay rats were not different. The bolus injection of atrial extract caused significant increases in urine volume and sodium excretion in both R and S rats. This increase in excretion was seen by 10 minutes, and by 20 minutes the excretion of urine and sodium had returned to control levels. Furthermore, ten

FIGURE 18. Comparison of EC_{50} values for norepinephrine-induced contractions, of normal rat aortic rings prior to, and following application of atrial extract from Dahl R rats consuming low (0.4% NaCl) or high (8.0% NaCl) salt diets. Final bath concentration of extract was 40 micrograms atrial tissue per ml. All values are group mean $\pm$ SE.

EFFECTS OF ATRIAL EXTRACTS FROM R RATS ON LOW AND HIGH SALT DIETS ON THE EC_{50} FOR NOREPINEPHRINE IN NORMAL RAT AORTIC RINGS

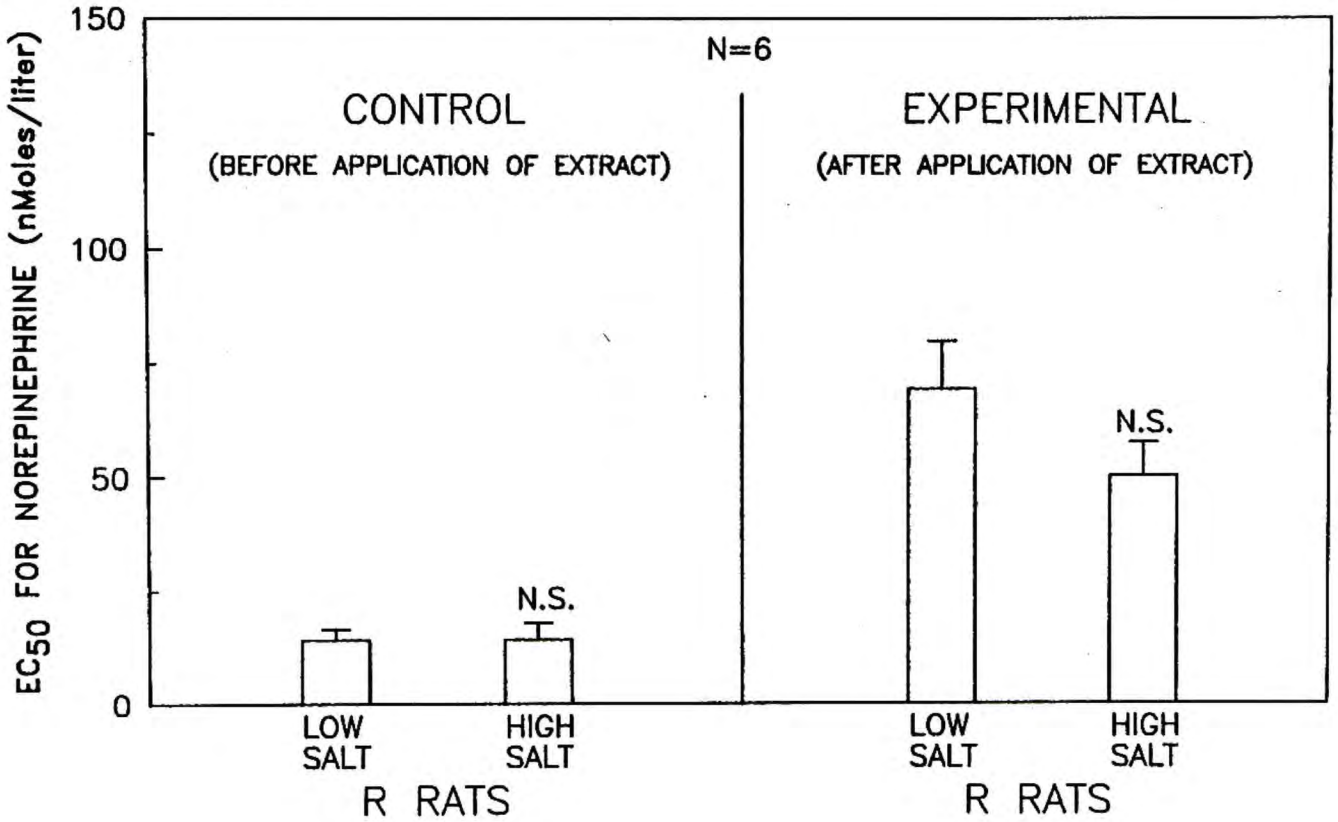


FIGURE 19. Comparison of EC_{50} values for norepinephrine-induced contractions, of normal rat aortic rings prior to, and following application of atrial extracts from Dahl S rats consuming low (0.4% NaCl) or high (8.0% NaCl) salt diets. Final bath concentration of extract was 40 micrograms atrial tissue per ml. All values are group mean ± SE.

EFFECTS OF ATRIAL EXTRACTS FROM S RATS ON LOW AND HIGH SALT DIETS ON THE EC_{50} FOR NOREPINEPHRINE IN NORMAL RAT AORTIC RINGS

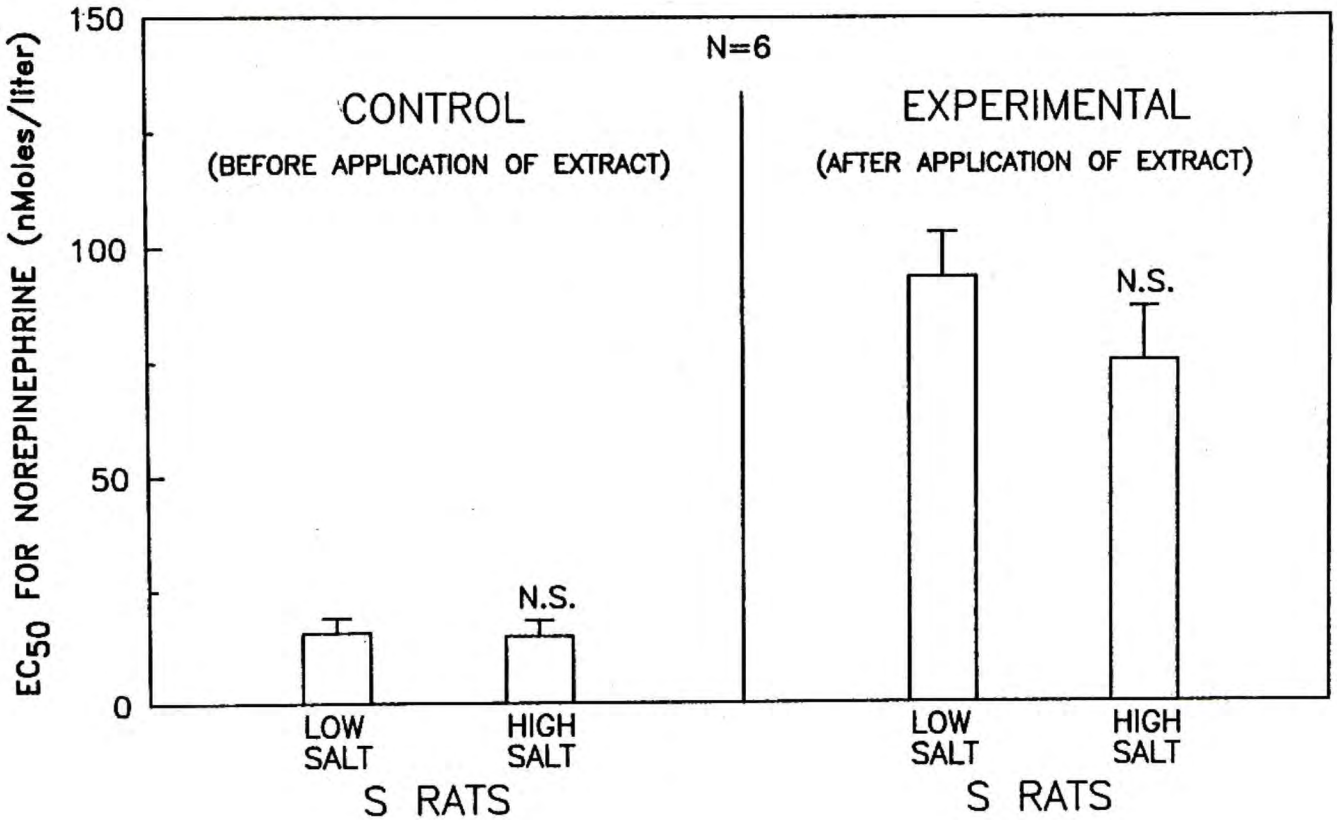
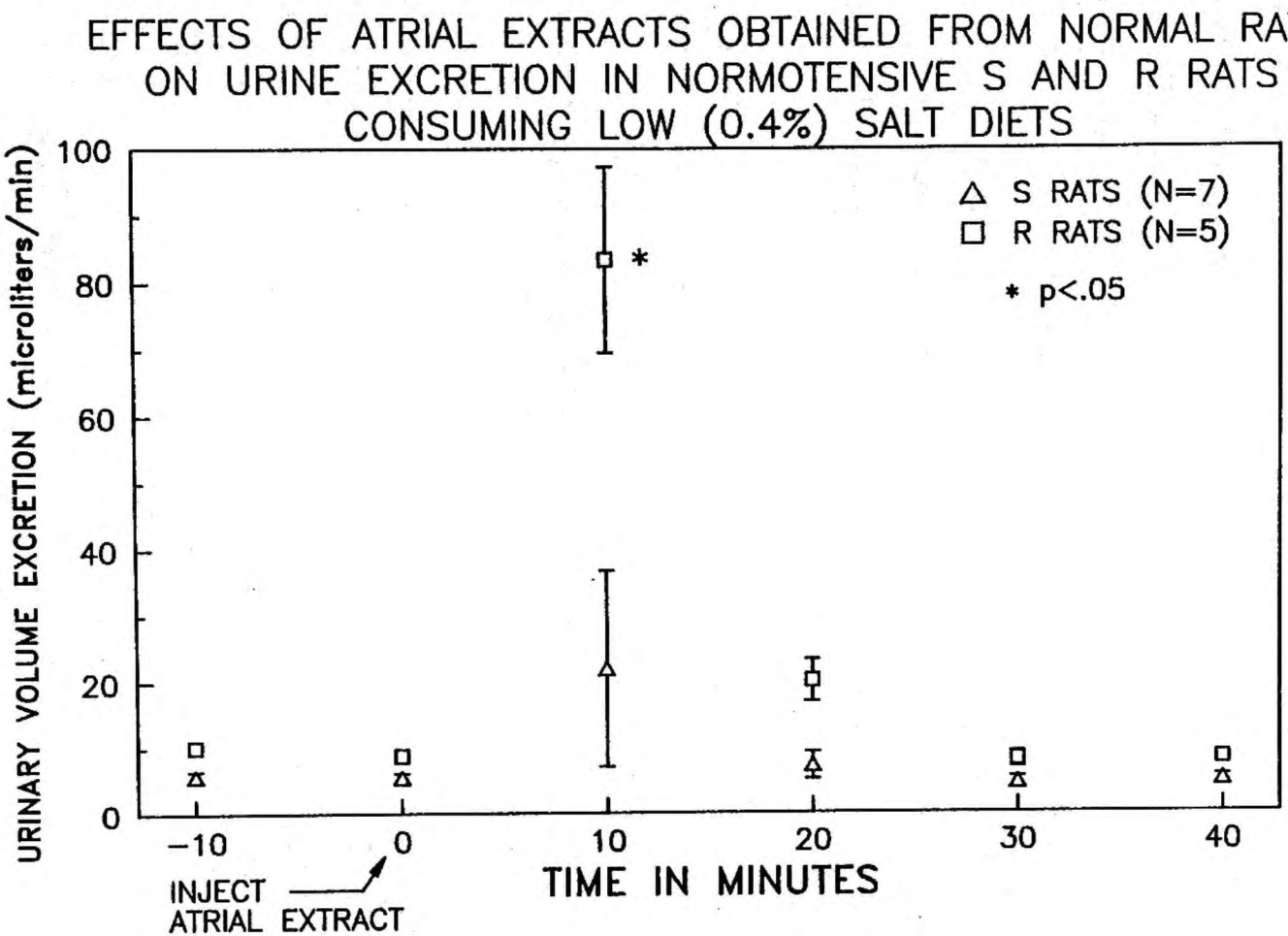


FIGURE 20. Urinary volume excretion (UV) of normotensive Dahl S and R rats, on low (0.4% NaCl) salt diets, prior to, and following i.v. injection of 0.20 ml of atrial extract obtained from normal Sprague Dawley rats. All values are group mean ± SE.


EFFECTS OF ATRIAL EXTRACTS OBTAINED FROM NORMAL RATS
ON URINE EXCRETION IN NORMOTENSIVE S AND R RATS
CONSUMING LOW (0.4%) SALT DIETS
URINARY VOLUME EXCRETION (microliters/min)
100
80
60
40
20
0
S RATS (N=7)
R RATS (N=5)
* p<.05
*
−10
0
10
20
30
40
INJECT
ATRIAL EXTRACT
TIME IN MINUTES

minutes following injection of 0.20 ml of normal rat atrial extract, R rats showed approximately four times greater urine volume excretion relative to identically treated S rats (83.4 ± 13.9 vs. 21.9 ± 14.8 microliters/min respectively). The difference between the strains was not significant at 20 minutes post-injection nor different from control levels. At 30 minutes the responses were not different from control pre-injection levels.

Urinary sodium excretion of these same S and R assay rats is presented in Figure 21. Prior to injection, the S and R assay rats were not different in their sodium excretion. Ten minutes after i.v. injection of the normal rat atrial extract, R rats showed four times greater sodium excretion than identically prepared S rats (10.0 ± 2.0 vs. 2.4 ± 2.0 microequivalents Na^+/min respectively). The difference was not apparent at 20 minutes post-injection.

2. Vascular responsiveness of Dahl S and R rats to atrial extracts.

Figure 22 represents the contractile responses of aortic rings obtained from normotensive Dahl S and R rats on low (0.4% NaCl) salt to the addition of atrial extracts from normal Sprague Dawley rats to the contractility bath. The percent change in the EC_{50} for norepinephrine of these aortic rings is plotted against the concentration of atrial extract. The aortic rings from the Dahl S and R rats were not different in their responsiveness to norepinephrine prior to the addition of the normal atrial extract (EC_{50} values were 15.2 ± 1.7 and 9.7 ± 1.3 nM for S and R rats respectively). Following addition of the atrial extract, approximately threefold greater responses were observed in aortic rings from R rats relative to those from S rats (1478 ± 276 vs. 436 ± 60%

FIGURE 21. Urinary sodium excretion ($U_{Na}V$) of normotensive Dahl S and R rats, on low (0.4% NaCl) salt diets, prior to, and following i.v. injection of 0.20 ml of atrial extract obtained from normal Sprague Dawley rats. All values are group mean ± SE.

EFFECTS OF ATRIAL EXTRACTS OBTAINED FROM NORMAL RATS ON SODIUM EXCRETION IN NORMOTENSIVE S AND R RATS CONSUMING LOW (0.4%) SALT DIETS

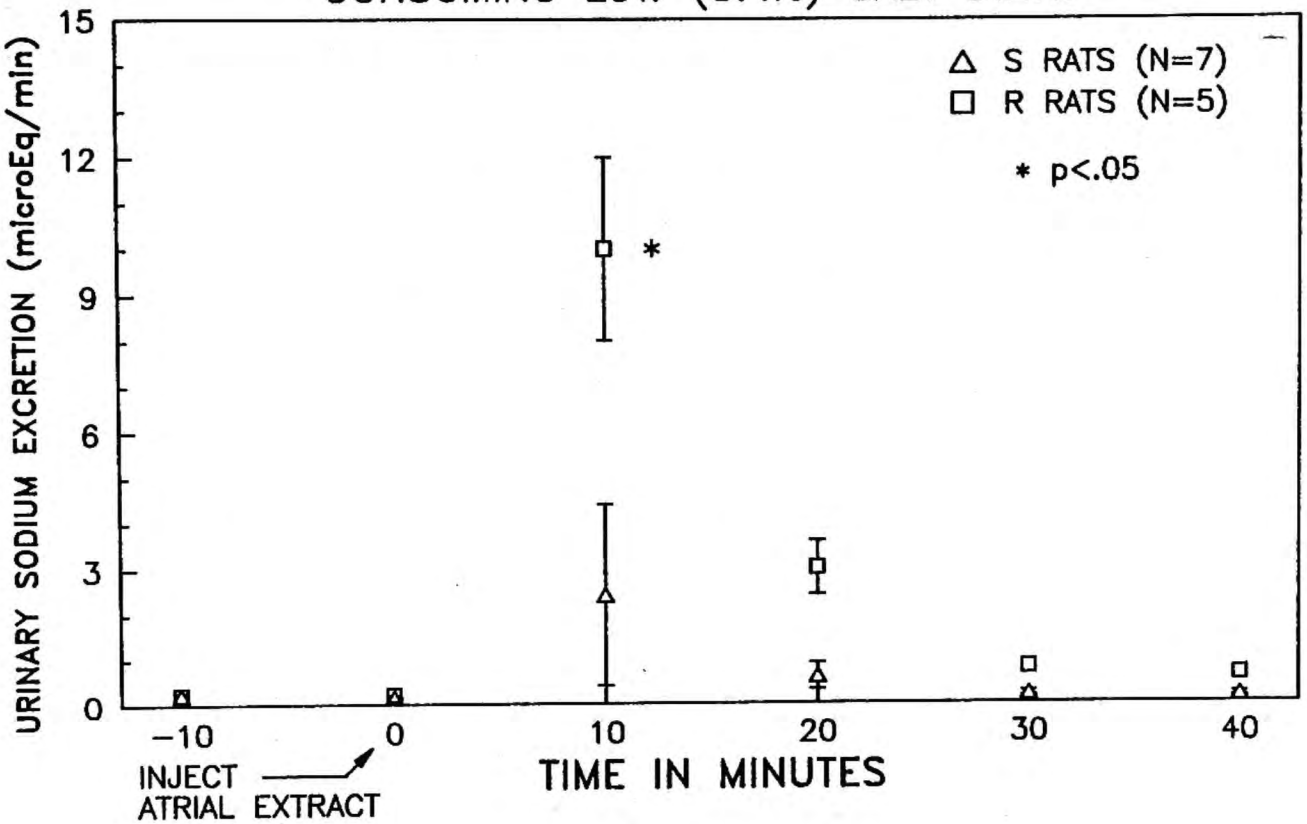
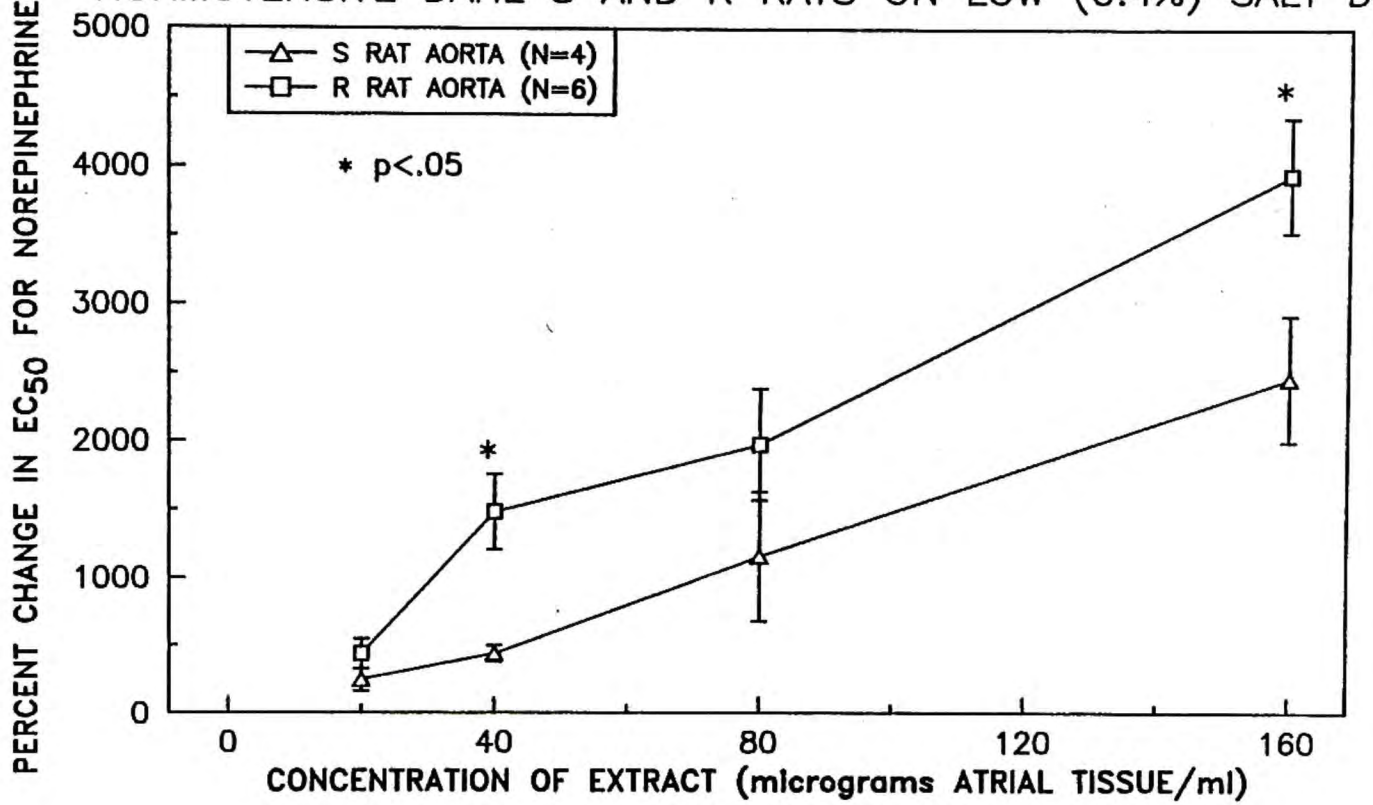


FIGURE 22. Percent change in the EC_{50}, for norepinephrine-induced contrtactions, of aortic rings from normotensive Dahl S and R rats consuming low (0.4% NaCl) salt diets, following appllication of various concentrations of atrial extract from normal rats. All values are group mean ± SE.

EFFECTS OF ATRIAL EXTRACTS PREPARED FROM NORMAL RATS ON THE RESPONSE TO NOREPINEPHRINE IN AORTIC RINGS FROM NORMOTENSIVE DAHL S AND R RATS ON LOW (0.4%) SALT DIETS



increase respectively) at a bath concentration of extract of 40 micrograms atrial tissue/ml, suggesting that the S rat is less sensitive to the vasorelaxant effects of ANF than similarly treated R rats at this concentration of atrial extract. Significant differences were also detected at 160 micrograms/ml where atrial extract increased the EC_{50} for norepinephrine by 3940 ± 416% in R rat aortae while only increasing that of S rat aortae by 2457 ± 462%. The difference in responses at the 80 microgram/ml concentration was not significant.

DISCUSSION

THE DAHL MODEL OF HYPERTENSION

Rats consuming grain-based diets

Our results, as expected, show that increasing salt (8.0%) in the diet results in the development of hypertension in Dahl S, but not Dahl R rats (Figure 4). S rats on low salt (0.4%) do show significant elevation of systolic blood pressure relative to similarly fed R rats. These results are in agreement with those of several other investigators and demonstrate that the salt sensitivity of S rats exists even at NaCl concentrations considered to be quite low. Dahl et al. (1968) reported mild hypertension in some individual S rats at dietary NaCl concentrations of 0.15%.

Body weights were not significantly different among the four groups of Dahl rats at any time during feeding with the grain-based dietary regimen (Table 1). Exposure to high salt in either strain, or the development of hypertension in S rats on high salt did not significantly alter body weight in these studies.

Rats consuming synthetic sodium-deficient diets

Our findings suggest that substitution of a sodium-deficient, synthetic diet for the grain-based diet used in all our other studies did not alter the development of hypertension in S rats when 8.0% NaCl

was added to the diet. S rats became hypertensive when fed this 8.0% NaCl diet while R rats, fed similarly, remained normotensive and not significantly different from R rats fed the sodium-deficient diet (Figure 6). The blood pressure of S rats fed the synthetic (0.04% NaCl) diet was not different from R rats at the start of the observation period (seven weeks of age). This is in contrast to the blood pressure of S rats, consuming the grain-based (0.4% NaCl) diet, which was significantly different from R rats at this age. However, the blood pressure of S rats on the synthetic diet did rise to 133 $\pm$ 1 mmHg by 14 weeks of age and at this time was not different from S rats consuming the grain-based diet. These findings suggest that the higher blood pressure of S rats, relative to R rats when both were on low salt and at 14 weeks of age, may be age related and further suggests that sodium deprivation in S rats may delay this response.

Our data on the body weights of Dahl S or R rats consuming the synthetic diet (Figure 5) shows that their body weights were significantly lower than those of S or R rats consuming grain-based diets. These differences were present at any time period in the study. Furthermore, S or R rats fed the synthetic diet with 8.0% added NaCl for five weeks had higher body weights than rats of the same strain consuming the synthetic diet without added NaCl. These findings suggest that the synthetic sodium deficient diet may have NaCl levels lower than that required for normal growth or may be lacking in some other necessary nutrient. In addition, there is the possibility that the higher weight in rats consuming high salt may be due to volume expansion. However, this weight gain is present in R rats that do not become volume expanded when fed high salt.

THE OUABAIN-LIKE HUMORAL FACTOR (OLHF)

Other investigators have reported depressed vascular $Na^+ - K^+$ pump activity in cardiovascular cells and the presence of a ouabain-like humoral factor (OLHF) in plasma of several forms of experimental hypertension (Huot et al.,1983; Pamnani et al., 1981). All these models of hypertension were low renin with presumed or documented volume expansion. Since $Na^+ - K^+$ pump inhibition can produce vasoconstriction by causing depolarization of vascular smooth muscle cells, increased intracellular Ca^{++}, and increased responsiveness to vasoconstrictor agents (Haddy et al., 1976) or increased intracellular Ca^{++} secondary to alterations in Na^+/Ca^{++} exchange (Blaustein, 1977), it has been suggested that pump inhibition by this circulating factor may be an underlying cause in the development and maintenance of hypertension in these models (Haddy et al., 1976).

In contrast to the findings of the presence of this $Na^+ - K^+$ pump inhibiting factor in the above models we found no evidence for the existence of a similar factor in the plasma of the Dahl strain hypertensive S rat which is also a low renin model (Iwai et al., 1973 and Tobian et al., 1978). The lack of the presence of this factor in this model is also suggested by the findings of Pamnani et al. (1980) and Overbeck et al. (1981) both of whom reported increased rather than decreased vascular $Na^+ - K^+$ pump activity in hypertensive Dahl S rats on high salt as compared to their normotensive R rat controls on high salt.

In the present study normal tail arteries incubated in boiled plasma supernates from S or R rats on low (0.4%) or high (8.0%) salt diets all showed similar ^{86}Rb uptake (Figure 7). If the factor were present in the plasma of the hypertensive S rat, then their plasma

supernates should have inhibited the vascular $Na^+ - K^+$ pump activity of these tail arteries from normal rats relative to arteries incubated in plasma from normotensive control rats. The methods used in preparation of the plasma supernates and the ^{86}Rb uptake method were identical to those used by Pamnani et al. (1981) and Huot et al. (1983) to demonstrate the presence of this factor in other hypertension models. The plasma supernates did not differ in any of the measured constituents except that the protein content of supernates from S rats on high salt was greater than that from the other three groups. This difference cannot be associated with elevation of OLHF content of these supernates since the ^{86}Rb uptake bioassay demonstrated there was no difference in OLHF content among the four groups.

However, the absence of increased levels of OLHF in hypertensive Dahl S rats on high salt may have been due to the diet these rats were consuming. It is possible that S rats on low (0.4%) salt diets may have been volume expanded despite this restriction of their salt intake. The previously mentioned studies of Tobian et al. (1978), Girardin et al. (1980) and Maude and Kao Lo (1982) have demonstrated decreased natriuretic capacity of the kidneys of S rats, predisposing them to salt retention and volume expansion. If this were the case, the S rat consuming low salt may have had the ouabain-like factor in its plasma, in response to this volume expansion, at levels sufficient to mask any difference in Rb uptake of normal tail arteries incubated in plasma supernates from the two groups of S rats, but not sufficient to cause hypertension in S rats consuming low salt. However, this does not appear to be the case, as there was no difference in Rb uptake of tail arteries incubated in plasma supernates from hypertensive S rats on high

salt relative to supernates from R rats on high salt. R rats are resistant to salt retention and volume expansion and should therefore not have had elevated levels of OLHF in their plasma after consuming high salt diets. The lack of a difference in tail artery Rb uptake following incubation in plasma supernates from these two groups of rats therefore suggests that the plasma levels of OLHF in S rats is also not elevated. Additionally, to rule out the possible effect of increased volume in S rats consuming 0.4% NaCl diets, on the release of OLHF, in another group of Dahl rats, we substituted a synthetic sodium-deficient diet (0.04% NaCl) for the normal low salt (0.4%) diet. S rats on this extremely low dietary salt intake should not be volume expanded, yet again there was no difference in Rb uptake in normal tail arteries when incubated in plasma supernates from these rats relative to supernates from hypertensive S rats on the same diet containing 8.0% added NaCl. This, again, suggests the absence of elevated levels of OLHF in these hypertensive S rats.

These experiments therefore suggest that there is no elevation of OLHF in the established phase of hypertension (4 to 6 weeks) in S rats and that it probably does not play a role in the pathophysiology of the high blood pressure in this hypertensive model. However, these experiments do not rule out the presence of elevated levels of OLHF in the initial phases of this hypertension. That it may in fact be elevated in the initial phase was demonstrated by Paschal et al. (1985) who showed elevation of red blood cell intracellular sodium concentration in S rats compared to R following 12 - 14 days of high salt intake. The authors suggested that this may be due to depressed $Na^+ - K^+$ pump activity secondary to increased plasma levels of a

digitalis-like factor.

The above study suggests that OLHF may be involved in the development of hypertension in this model. However, our findings suggest that it is not involved in the maintenance of hypertension once established. Therefore, the maintenance of elevated blood pressure in the established phase is due to some mechanism(s) other than elevation of plasma OLHF. An alternate mechanism of hypertension in Dahl S rats may involve changes in the levels of, and the cardiovascular and renal effects of Atrial Natriuretic Factor.

ATRIAL CONTENT OF ANF IN THE DAHL STRAIN

Changes in body fluid volume have been shown to affect the density of atrial granulations in rats (de Bold et al., 1979) although the significance of these changes is not clear. Water deprivation lead to atrial hypergranulation while volume loading caused by DOCA lead to degranulation. The relationship of atrial granularity to circulating ANF levels remains to be clarified. Atrial ANF levels have also been shown to be altered in experimental models of hypertension (Sonnenberg et al., 1983 and Pamnani et al., 1984b). In the present study we measured the level of ANF in the atria of hypertensive Dahl S rats and their controls to determine if altered atrial content of ANF may be involved in the hypertension in this model.

Natriuresis-Diuresis bioassay of atrial ANF content

The significantly greater natriuresis and diuresis of normal assay rats following intravenous injection of atrial extracts prepared from hypertensive S_{HS} rats compared to those from R_{HS} rats suggests higher levels of ANF in S rats on high salt relative to R rats on high salt. Similarly, greater levels of ANF were also seen in atrial extracts prepared from normotensive S rats on low salt relative to R rats on the same low salt diet, suggesting that a difference in atrial ANF content exists between the two strains that is not dependent on salt intake or the presence of hypertension in S rats. Additionally, atrial levels of ANF were also higher within strains when comparing rats consuming high salt to those on low salt diets. This suggests that atrial levels of ANF may be elevated by increased salt intake per se.

These findings are in agreement with those of Hirata et al. (1984) where greater atrial ANF content was demonstrated by natriuresis bioassay in S rats, following consumption of 4.0% NaCl for 12 weeks, relative to R rats on the same dietary regimen. Furthermore, they reported increased atrial ANF in both strains following five days on the diet. By 12 weeks, the atrial content of ANF had returned to control levels in R rats but remained elevated in S rats. These results in R rats were not observed in our study, probably because we did not continue the dietary regimen for more than six weeks. Greater atrial content of ANF was also demonstrated in S rats, relative to R, when both were maintained on diets containing 0.11% NaCl for 12 weeks. Our findings after six weeks of 0.4% NaCl diets are in agreement with this observation and further suggest that a genetic difference in atrial ANF content exists between the two strains prior to exposure to high salt

diets or the development of hypertension in S rats.

Radioimmunoassay of atrial ANF content

Our results from the studies involving radioimmunoassay of atrial extracts from Dahl rats also suggest that the ANF content of atria from hypertensive S rats on high salt is greater than that of R rats on high salt, thus confirming our natriuresis bioassay results. However, unlike the bioassay, the radioimmunoassay showed no difference between the strains when both consumed low salt diets, or within either strain when comparing rats consuming high salt relative to those consuming low salt. Our findings in Figure 12 were of greater AP III content in R rats on low relative to high salt, although this difference was not significant. It is our feeling that the values for AP III content in the R_{LS} rats were abnormally high and that a larger sample size would have explained this discrepancy. Schwartz et al. (1985a) reported no difference, as determined by radioimmunoassay, between S and R rats when consuming high or low salt diets. Furthermore, these authors reported decreased atrial ANF levels in both strains when consuming high, relative to low, salt diets. These results are in contrast to our findings and this discrepancy could be due to the radioimmunoassay procedure. The specificity of the antibodies used were not reported. The antibodies used may have been raised against different fractions of atrial extracts or against different fragments of ANF. It is likely that differing specificities of the antibodies, and techniques used in extracting the ANF from the atria could have accounted for these differences. Snajdar and Rapp (1985) reported that

atrial ANF levels, as determined by radioimmunoassay and natriuresis bioassay, were greater in S, relative to R rats when both were on low salt diets, at one and two months of age. The specificity of the antibodies in this study were however not reported either.

The radioimmunoassay procedure in our studies used rabbit antibodies directed against the C terminal heptapeptide of AP III. Hom et al. (1985), in our laboratory, demonstrated that AP iii accounted for only 15% of the immunoreactivity in atrial extracts. The antibody is specific for atriopeptins containing a C terminal TYR residue and therefore should not detect AP I or AP II. In addition, it was shown that different extraction techniques used on the atria resulted in differing yields of this AP III-like immunoreactivity (Hom et al., 1985). Extraction in 0.1 N acetic acid or boiling in phosphate-buffered saline yielded smaller amounts of AP III-like immunoreactivity relative to extracts made in 1.0 N acetic acid. In our studies, 0.1 N acetic acid extracts were used in the natriuresis-diuresis bioassay, while extracts made in 1.0 N acetic acid were used in the radioimmunoassay. It is possible that these differences in preparation could have resulted in differences in the relative concentrations of the various atriopeptins in the extracts. AP I, AP II and AP III have all been shown to cause natriuresis (Currie et al., 1984 and Geller et al., 1984) and should all be detected by the natriuresis bioassay, whereas our radioimmunoassay detects only AP III and other TYR-containing atriopeptins. It is possible that the differences in ANF content, as determined by the natriuresis bioassay, of S and R rats consuming low salt diets, and the elevation of ANF within strains when comparing rats consuming high relative to low salt diets, may have been due to some

form of atriopeptin other than AP III, as these differences were not detected by our radioimmunoassay. In the study by Schwartz et al. (1985a) the extraction procedure was not reported, and a difference in the procedures may explain why their results are not in agreement with ours.

Contractility bioassay for atrial ANF content

Our results from contractility bioassay of atrial extracts from Dahl S and R rats also suggest that atrial ANF levels are higher in S rats relative to R rats. Atrial extracts from S rats on high salt caused a greater inhibition of norepinephrine-induced contractions of normal rat aortic rings than extracts from R rats on high salt. This difference was also present when both rats consumed low salt diets. These findings are in agreement with our results from the natriuresis bioassay. The differences in ANF content within strains when comparing rats consuming high relative to low salt diets were not detected in this study. This apparent discrepancy may be due to differences in sensitivity of the two bioassay systems. It has been suggested that the renal actions of ANF involve more than the renal vasculature as direct renal tubular effects had been demonstrated (Sonnenberg et al., 1981b and Briggs et al., 1982). Therefore it is not surprising that a bioassay system using the rat aorta may behave differently than one using the rat kidney.

The sensitivity of the two bioassay systems used, smooth muscle contractility and natriuresis, probably are different. The first measures a purely vascular effect while the latter system detects a

combination of renovascular and tubular effects. When intravenously injected, the natriuretic effect is much greater than the effect on blood pressure. In addition, the natriuresis bioassay is performed in vivo and may therefore be more relevant physiologically than the in vitro contractility assay. The various forms of the peptide also seem to have differing potencies, depending on the tissue on which they are tested. AP II causes four times greater natriuresis than AP I (Currie et al., 1984) but the potency of AP III relative to AP I and AP II has not been evaluated.

We examined the relative potencies of AP I, AP II and AP III in our contractility bioassay. Greater inhibition of norepinephrine-induced contractions was achieved using AP III compared to AP I and AP II. AP I showed the least activity, and was approximately one half as potent as AP III in this inhibitory effect. The order of potency was AP III > AP II > AP I, where AP III increased the EC_{50} (decreased the responsiveness) for norepinephrine approximately tenfold relative to control. These results are consistent with those of Garcia et al. (1984) in which the necessity, for vasorelaxant activity, of the PHE-ARG carboxyl residues (present on AP II and AP III, but not AP I) was demonstrated. Wakitani et al. (1985) also showed that this PHE-ARG residue was necessary for renal vasodilator activity. Since AP I has been shown to have little vasorelaxant activity, our contractility bioassay will probably not detect this form of atriopeptin in atrial extracts assayed by this technique. In contrast to the vasorelaxant effect, natriuresis and diuresis is induced by all three atriopeptins. Because the natriuresis bioassay depends on the activity of all three of these peptides, it is probably more sensitive than the contractility

bioassay which does not recognize atriopeptins lacking this PHE-ARG carboxyl residue (e.g. AP I). This greater sensitivity may explain why the natriuresis bioassay detected differences between Dahl atrial extracts that were not detected by the other assay systems used.

Our findings using the above three assay systems consistently detected higher levels of ANF in the atria of hypertensive S rats relative to R rats when both were consuming high salt diets. The two bioassay systems (natriuresis and smooth muscle contractility) also detected this difference between the strains when both strains consumed low salt diets and the S rats remained normotensive. The absence of this finding in the radioimmunoassay study may be due to the specificity of the antibody used and therefore the sensitivity of the assay system. Snajdar and Rapp (1985) did detect this difference between the strains, while the rats were fed low salt, using their own radioimmunoassay procedure, but the details of their methods were not provided. Unique to our natriuresis bioassay was the findings of increased atrial ANF in both S or R rat strains in response to high salt intake. These findings were not confirmed by our radioimmunoassay or contractility bioassay and are therefore less conclusive. Schwartz et al. (1985a) reported opposite findings: a decrease in atrial ANF content, following high salt feeding, at two and twelve weeks. We did not measure ANF levels at these time periods and are unable to explain this discrepancy without further information on their assay system.

RESPONSIVENESS OF DAHL S AND R RATS TO EXOGENOUS ANF

Our studies indicate that normotensive S rats on low salt show markedly less natriuresis and diuresis than R rats on low salt when injected with equal quantities of atrial extract from normal rats. This suggests that the renal responsiveness or sensitivity to ANF is depressed in S relative to R rats. Similar findings were reported by Hirata et al. (1984). This difference in renal responsiveness exists without prior exposure to high dietary salt intake or the presence of hypertension in S rats, and suggests a genetic abnormality that could predispose these rats to salt retention, extracellular fluid volume expansion and, possibly hypertension.

Similarly, we found that aortae from normotensive S rats on low salt show higher contractile responses to norepinephrine, in the presence of atrial extracts, than do similarly treated R rat aortae. These findings suggest that the antagonism of norepinephrine-induced contractions due to ANF, as shown in our studies of normal rat atrial extracts and synthetic atriopeptins and the studies of Kleinert et al. (1984), is reduced in S rats relative to R rats. At concentrations of 40 and 160 micrograms atrial tissue per ml, aortae from S rats had significantly lower EC_{50} values (greater responsiveness to norepinephrine) than similarly treated aortae from R rats. Similar studies have not been reported from other laboratories with respect to the Dahl strain of rats.

Other investigators (Irizawa et al., 1985; Chinn and Hartle, 1985) have reported decreased renal responsiveness to ANF in DOCA-saline and perinephretic hypertensive rats respectively. However, these studies were performed in hypertensive rats while our study demonstrated

this decreased responsiveness to ANF in the absence of hypertension. All Dahl strain rats used in our study for responsiveness to ANF were fed low salt (0.4% NaCl) diets from weaning. In this manner, additional variables such as hypertension, vascular restructuring and alterations in renal hemodynamics were avoided in S rats.

Our findings of decreased renal and vascular responsiveness to ANF in Dahl S rats suggests that altered responsiveness to ANF could be involved in the pathogenesis and maintenance of hypertension in S rats once exposed to high dietary salt. Decreased renal responsiveness to the natriuretic effects of ANF may be a factor that predisposes the S rats to salt retention and volume expansion by compromising their ability to excrete the increased salt load due to the high salt diets. The kidneys of S rats have an inherent defect in the ability to excrete salt (Tobian et al., 1978). The defect is present even in isolated, artificially perfused kidneys (Maude and Kao Lo, 1982) and therefore is not dependent on ANF. This inherent defect, coupled with the decreased responsiveness to the natriuretic effects of ANF, could lead to even greater salt retention and volume expansion.

Decreased vascular responsiveness to ANF could also predispose S rats to hypertension. In the presence of equal amounts of ANF, aortae from S rats show greater contractile responses to norepinephrine than do aortae from R rats. This difference in responsiveness also exists without prior exposure to high salt or the presence of hypertension. Since the effect of ANF seems to antagonize the vasoconstrictor response to norepinephrine in vitro, a decreased responsiveness to ANF could result in abnormally high sensitivity to norepinephrine in vivo. If ANF is to decrease peripheral vascular resistance in vivo, it would have to

have an effect on resistance vessels similar to that shown in the aorta. Osol and Halpern (1985) reported that synthetic ANF did not cause direct vasodilation of smaller (less than 300 micron) blood vessels. However, these results do not rule out ANF antagonism of endogenous catecholamine-induced contractions because these vessels were studied in the absence of catecholamines. ANF may have an effect on resistance vessels because decreased blood pressure in response to injection of ANF, although not very marked, have been reported in intact animals (de Bold et al., 1981: Sonnenberg et al., 1982; Borenstein et al., 1983; and Maack et al., 1984). The mechanism of vasodepressor activity in vivo is at present not known. Other investigators have reported that the decreased blood pressure is due to decreased cardiac output while peripheral resistance is increased (Kleinert et al., 1985; Natsume et al., 1985; and Brandt et al., 1985). The effect of ANF on resistance vessels and peripheral vascular resistance is therefore not clear at this time.

THE ROLE OF ANF IN CONTROL OF BLOOD PRESSURE

By nature of its biological actions, ANF can be considered to be antihypertensive. Its role in causing natriuresis and diuresis, and relaxation of vascular smooth muscle preparations have been discussed. In addition, intravenous injection has been reported to lower blood pressure in experimental animals. Alterations in ANF levels could be involved in hypertension in several different ways. Decreased levels of ANF could be involved, as a decrease in an antihypertensive substance could be considered pro-hypertensive. A decrease in the sensitivity to

ANF would also lead to decreased antihypertensive activity and could promote the development of hypertension. Increased levels of ANF, could also be the result of a conpensatory response to the hypertension itself.

Our findings of increased atrial levels of ANF in hypertensive S rats on high salt, relative to R rats on high salt, suggest that ANF may be involved in the hypertension seen in S rats. It is not clear what these atrial levels mean. They could represent increased synthesis accompanied by even greater release, or decreased release alone. These findings suggest that the hypertension seen in S rats is not due to deficiency in ANF levels. However, S rats appear to be less sensitive to ANF, a factor that could predispose these rats to hypertension, as discussed. The increased levels could therefore be a compensatory response to the decreased renal and vascular sensitivity to ANF. It is possible that, in the absence of this compensatory response, the hypertension in the S rats would be of a greater magnitude.

It is also possible that the atrial levels of ANF do not reflect the levels circulating in the plasma. If ANF is a true hormone, it must be released into the circulation to act on target organs or tissues, and therefore it would be the plasma levels of ANF and its turnover in the tissues that was of physiological significance. Whether or not there is an alteration of the plasma levels of ANF in the hypertensive Dahl S rat has not been determined at this time. Radioimmunoassay procedures with sufficient sensitivity to detect ANF levels in plasma have been developed only recently. The problems of the antibodies not recognizing all the atriopeptins and the implications in interpretation of the data have been discussed.

Several lines of evidence suggest that ANF is a true hormone. Its release into the circulation following volume expansion and atrial stretch have been demonstrated (Dietz et al., 1984 and Lang et al., 1985). ANF apparently also modulates the action of other hormonal systems. Its release is stimulated by vasopressin (Manning et al., 1985) and it reciprocally inhibits vasopressin release (Samson, 1985). In addition, ANF inhibits renin and aldosterone secretion (Burnett et al., 1984; Vari et al., 1985; and Atarashi et al., 1984). Recent studies by Schwartz et al. (1985b) suggest that the SER-LEU-ARG-ARG-AP III form is the major circulating form of the peptide. If this is the case, then the AP I, AP II and AP III-like activities demonstrated in our studies, and by other investigators, may be breakdown products of this form of atriopeptin as the result of enzymatic degradation or breakdown during the extraction from atrial tissue. Further studies are needed to evaluate the role of this form of atriopeptin in the Dahl model of hypertension.

SUMMARY AND CONCLUSIONS

We have presented data from our own, and other laboratories that suggest that altered levels of the ouabain-like humoral factor (OLHF) and atrial natriuretic factor (ANF) may be involved in the development and maintenance of hypertension in several experimental models. We have extended these studies to the Dahl strain of Salt-Sensitive and Salt-Resistant rats. Our results suggest that OLHF is probably not involved in the established phase of hypertension in this model. Using methods identical to those in studies where the presence of OLHF was demonstrated, we were unable to find evidence for its presence in this form of hypertension. Evidence for the presence of increased levels of OLHF in the development phase of hypertension in the Dahl strain has been reported by another investigator, and therefore a role for this natriuretic and antinatriferic substance in the development of hypertension in this model cannot be ruled out at this time without further studies.

ANF, through its diuretic/natriuretic and vasorelaxant effects, has antihypertensive actions. Studies from our laboratory, and others, have suggested that ANF may also be involved in the development and maintenance of some forms of experimental hypertension. Results of this study show increased atrial tissue levels of ANF in hypertensive S rats. Furthermore, our data shows decreased renal and vascular sensitivity to ANF which may be a condition predisposing these rats to hypertension. Decreased renal sensitivity to ANF may be a factor predisposing Dahl S rats to salt retention and resultant expansion of extracellular fluid volume. Decreased vascular sensitivity to ANF, by attenuating its

antagonism of the action of vasoconstrictors (e.g. norepinephrine) may allow greater expression of the action of these vasoconstrictors. Since sympathetic nerve activity has been reported to be greater in hypertensive S rats relative to R rats (Takeshita and Mark, 1978), decreased vascular responsiveness to ANF, if present in these rats, should augment the functional vasoconstrictor action of this increased sympathetic activity. The increased vascular resistance and hypertension in S rats on high salt may therefore be due, at least in part, to this mechanism and we feel that this possibility cannot be ruled out at this time. The higher levels of atrial ANF, if representative of circulating plasma levels, may be a compensatory response to the decreased responsiveness of their kidneys and vasculature to the antihypertensive effects of ANF and may play a role in modulation of blood pressure levels in these rats.

Several new questions have arisen as a result of these studies. Further investigation is necessary to determine whether atrial tissue levels of ANF are correlated with circulating levels in the plasma and to determine the free circulating concentration. In addition, detailed information is required regarding the possible time-dependent changes in ANF and OLHF during development of the hypertension, and during the established phase. The renal and vascular responsiveness to OLHF and ANF need quantitative evaluation under similar conditions. Some of such future studies may become especially relevant if the isolation and chemical characterization of OLHF can be achieved, for example the development of specific antibodies and antagonists to OLHF. Similar developments regarding ANF would also be necessary to clarify its role in the hypertensive process.

APPENDIX

TABLE 2. Group means ± SE of weekly systolic blood pressures of Dahl S and R rats consuming grain-based low (0.4% NaCl) and high (8.0% NaCl) salt diets.

Table 2

SYSTOLIC BLOOD PRESSURE OF DAHL S AND R RATS
CONSUMING GRAIN-BASED DIETS

	0.4% NaCl DIET		8.0% NaCl DIET		
WEEKS OF TREATMENT	R rats (N=27)	S rats (N=27)	R rats (N=33)	S rats (N=25)	p Value
-1	119 ± 1	129 ± 1*	120 ± 1	129 ± 2**	p<.05
0	118 ± 1	129 ± 1*	120 ± 1	129 ± 1**	p<.05
1	120 ± 1	130 ± 1*	120 ± 1	145 ± 2**,***	p<.05
2	119 ± 1	131 ± 1*	120 ± 1	155 ± 2**,***	p<.05
3	119 ± 1	131 ± 1*	120 ± 1	158 ± 2**,***	p<.05
4	120 ± 1	131 ± 1*	120 ± 1	164 ± 2**,***	p<.05
5	120 ± 1	132 ± 1*	120 ± 1	167 ± 2**,***	p<.05
6	120 ± 1	132 ± 1*	120 ± 1	170 ± 2**,***	p<.05

(all values in mmHg)

* S_{LS} relative to R_{LS}

** S_{HS} relative to R_{HS}

*** S_{HS} relative to S_{LS}

Table 3. Group means ± SE of weekly body weights of Dahl S and R rats consuming synthetic sodium-deficient (0.04% NaCl) diets, or synthetic diets containing 8.0% added NaCl.

Table 3

BODY WEIGHT OF DAHL S AND R RATS
CONSUMING SYNTHETIC SODIUM-DEFICIENT DIETS

	0.04% NaCl DIET		8.0% NaCl DIET		
WEEKS OF TREATMENT	R rats (N=7)	S rats (N=7)	R rats (N=7)	S rats (N=7)	p Value
-1	127 ± 4	123 ± 6	118 ± 7	111 ± 4	N.S.
0	138 ± 5	142 ± 7	133 ± 7	113 ± 6*	p<.05
1	142 ± 5	153 ± 9	158 ± 7	158 ± 5	N.S.
2	153 ± 8	164 ± 12	168 ± 6	175 ± 6	N.S.
3	173 ± 10	176 ± 13	178 ± 7	191 ± 7	N.S.
4	169 ± 13	194 ± 13	200 ± 9	221 ± 9	N.S.
5	191 ± 12	208 ± 15	236 ± 8**	266 ± 8*,***	p<.05
6	197 ± 8	218 ± 14	252 ± 8**	286 ± 7*,***	p<.05

(all values in grams)

* S_{HS} relative to S_{LS}

** R_{HS} relative to R_{LS}

*** S_{HS} relative to R_{HS}

TABLE 4. Group means ± SE of weekly systolic blood pressures of Dahl S and R rats consuming synthetic sodium-deficient (0.04% NaCl) diets, or synthetic diets containing 8.0% added NaCl.

Table 4

SYSTOLIC BLOOD PRESSURE OF DAHL S ANS R RATS
CONSUMING SYNTHETIC SODIUM-DEFICIENT DIETS

	0.04% NaCl DIET		8.0% NaCl DIET		
WEEKS OF TREATMENT	R rats (N=7)	S rats (N=7)	R rats (N=7)	S rats (N=7)	p Value
-1	119 ± 1	121 ± 1	119 ± 1	120 ± 1	N.S.
0	119 ± 1	121 ± 1	119 ± 1	119 ± 1	N.S.
1	119 ± 1	123 ± 1	118 ± 1	141 ± 1$^{*}_{**}$	p<.05
2	119 ± 1	127 ± 1***	118 ± 1	156 ± 1$^{*}_{**}$	p<.05
3	121 ± 1	130 ± 1***	118 ± 1	165 ± 1$^{*}_{**}$	p<.05
4	121 ± 1	132 ± 1***	119 ± 1	173 ± 1$^{*}_{**}$	p<.05
5	120 ± 1	131 ± 1***	118 ± 1	174 ± 2$^{*}_{**}$	p<.05
6	120 ± 1	133 ± 1***	118 ± 1	176 ± 1$^{*}_{**}$	p<.05

(all values in mmHg)

* S_{HS} relative to R_{HS}

** S_{HS} relative to S_{LS}

*** S_{LS} relative to R_{LS}

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